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This report describes the results of research in the following areas: Whether spontaneous or phytohemagglutinin-induced transformation of human lymphocytes in vitro is affected by exposure to continuous wave (CW) or pulsed wave (PW) 2450 MHZ microwaves. Conclusion: it is affect by PW in a non-thermal way. Comparison of CW and PW microwave exposure at the same average specific absorption rate (SAR) - PW is markedly different and acts in a non-heating way. Comparison of effects obtained by microwave and conventional heating needed to elevate the sample temperature by 0.5, 1.0, 1.5, and 2.0°C. Conclusion: CW microwaves and conventional heating produce the same effects, PW exposure behaves differently. To determine whether chromosomal aberrations are induced under the conditions of the experiment. Conclusion: they are not.

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EFFECTS OF PULSED AND CW 2450 MHZ RADIATION ON TRANSFORMATION AND CHROMOSOMES OF HUMAN LYMPHOCYTES IN VITRO

Final Progress Report to:

U.S. Army Medical Research and Development Command

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INTRODUCTION

The objectives of this project were:

- to ascertain whether the course of spontaneous or phytohemagglutinin (PHA)-induced blastic transformation of human lymphocytes *in vitro* is affected by exposure to continuous wave (CW) or pulsed (PW) 2450 MHz microwaves;
- to compare effects obtained by CW and PW microwave exposure at the same average specific absorption rate (SAR);
- to compare effects obtained by microwave heating and conventional heating needed to increase the temperature of the sample by 0.5, 1.0, 1.5 and 2.0°C.
- to ascertain whether chromosomal aberrations are induced under the conditions of these experiments.

In order to provide reliable quantitative data on exposure, a system with automated dosimetry was developed, and tested for biocompatibility with human lymphocyte cultures. A method for quantitation of lymphocyte transformation based on image analysis was developed and tested. Experiments on effects of CW or PW 2450 MHz exposure at heating and non-heating levels were carried out. Effects of conventional heating were examined and compared with effects of microwave heating. For convenience and clarity the work performed is described in the following sequence: (1) exposure system and dosimetry, and (2) studies on lymphocyte cultures, and (3) conclusions.

EXPOSURE SYSTEM AND DOSIMETRY

Description of the Exposure System

The experiments planned in this project necessitated the design and assembly of an exposure system that would meet several engineering, dosimetric and biological requirements. The system should provide:

(1) an environment compatible with lymphocyte culture in vitro in respect to relative humidity (90-100%), CO_2 content in air (5%), and temperature (37°C), as well as the possibility of heating and equilibrating the temperature of the samples up to 39°C using microwave or conventional heating;

(2) the possibility of exposing samples (preferably involving the exposure of multiple samples under identical conditions) to CW, or PW 2450 MHz with a high ratio of peak to average power, and the possibility of simultaneous sham exposure of control samples under strictly comparable conditions, where the only difference between exposed and sham exposed samples would consist in the presence (or absence) of microwave absorption in the sample;

(3) continuous measurements and registration of the temperature of exposed and sham exposed samples during exposure to provide a temperature profile over time history of the samples;

(4) continuous monitoring of the wave form and repetition rate in the case of PW exposures.

The first and second requirements were met by placing two shorted rectangular S-band waveguides 300 mm long (Omega Laboratories Models 6101) in a conventional CO_2 tissue culture incubator (Forma Scientific) thermostated at 37°C. A styrofoam block placed in the waveguide serves as support for the sample holder, and ensures that the sample is centrally placed in the waveguide in the same location in successive experiments. A small hole drilled in the waveguide allows the introduction of a minimally-perturbing temperature probe. The hole is drilled near the guide wall, at the center of the shorter dimension, this is a low electric field point and the hole produces minimal disturbance. One of the waveguides serves for sham exposures, the other one is connected through a matched coaxial cable to the microwave power source. This consists of several elements. The output from a CW Hewlett-Packard Model 8616A oscillator feeds a Hughes Model 1177H travelling wave tube amplifier. Pulsed or amplitude modulated waveforms can be obtained through the use of a Hewlett-Packard Model 8403A p-i-n modulator, for high peak power pulsed exposures, the signal can be further amplified with a 1 kW amplifier (MCL, La Grange, IL). The waveguide receives its power through an isolator and a dual directional coupler that allows forward and reflected powers to be measured with Hewlett-Packard Model 432A power meters and waveforms to be monitored using a Phillips PM 3240 oscilloscope. The amount of power supplied to the waveguide is controlled by the output from the oscillator and the

voltage of the 1 kW amplifier, and monitored by measurements of the forward and reflected powers, and by the readings on the oscilloscope. The main function of the oscilloscope is, however, to monitor waveforms. These means of control and monitoring are only auxiliary, as the conditions of exposure are characterized by dosimetry based on measurements of temperature and of SAR within the sample, as specified by the 3rd requirement the system should meet.

The dosimetric part of the system consists of two minimally-perturbing temperature probes, connected to a Hewlett-Packard Model 59306A relay activator, which is itself connected to a Keithley Model 192 digital voltmeter (DVM). Both the relay activator and the DVM are under the control of a Hewlett-Packard Model 86 desk-top computer through the HPIB (IEEE-488) bus. Initially a Narda Model 8011B non-perturbing double temperature probe was used, later two Vitek Model 101 probes were substituted for the Narda probe. The Narda probe requires cumbersome calibration against a NBS standard and introduction of correction factors to relate the current to temperature, a linear relationship being maintained over limited temperature ranges. Vitek probes are much easier to calibrate, are more stable, and the current-temperature relationship is linear over the temperature range of interest.

Under computer control the temperatures of exposed and sham exposed samples are recorded sequentially and stored in memory (records are maintained by keeping a library of micro-floppy disks). Since the temperature of the sham exposed sample is essentially constant in view of the stability of the temperature of the conventional incubator, it can be monitored less frequently than the temperature of the exposed samples. For conventional thermal exposures the temperature can be elevated by increasing the temperature of the incubator, maintaining control samples in another CO_2 incubator.

The sample holder has to be a tissue culture vessel made of materials non-toxic to cells *in vitro* and transparent to 2450 MHz microwaves. The geometry of the container has to allow for uniform energy deposition within the sample. These considerations limited the choice of possible sample holders. Previous experience with tissue culture exposures in a S-band waveguide demonstrated that the use of T-flasks or Petri dishes leads to signifi-

cant nonuniformities in energy deposition. Two types of sample holders were tested, both rectangular tissue culture Lab-Tek (Miles Laboratories) chamber slides, a two chamber model No4802, and a four chamber model No4804. Analysis of SAR distribution based on measurements of temperature (see below) in slide chambers containing tissue culture medium demonstrated that the energy distribution within and between chambers of the two chamber model is nonuniform, the difference between chambers being about 50%. In the four chamber model measurements demonstrated that differences in energy deposition remain within 10% of an average value. Therefore, the four chamber model was selected for use in experiments. Three of the chambers contain tissue culture medium and cells the fourth contains medium only. A hole is drilled in the cover of the chamber and a temperature probe is introduced. In this way each experiment can be carried out in triplicate on simultaneously exposed samples with simultaneous continuous dosimetric control.

Dosimetry and Temperature-Time Analysis

During an experiment the temperature in the exposed and the sham-exposed samples is recorded at regular intervals, with a minimum interval of less than 1 s. The temperature is recorded before the beginning of, during, and after the exposure. The "on" and "off" times of exposure are recorded on the computer by the operator using a soft-key interrupt capability. At the conclusion of a run the $T(t)$, i.e., temperature (T) versus time (t) behavior is analyzed to determine SAR.

If the SAR is S ($\mathrm{Wkg^{-1}}$) and the specific heat of the sample is $C(\mathrm{Jkg^{-1}K^{-1}})$, then the rate of heating during microwave exposure, for small temperature variations over which C can be assumed to be constant, is

$$\left(\frac{dT}{dt}\right) = \frac{S}{C} \tag{1}$$

If, in the absence of deliberate heating, a sample is above or below its equilibrium temperature, T_{eq}, with its surroundings, then for small temperature differences from equilibrium, its natural rate of temperature change, from Newton's law of cooling, is of the form

$$\left(\frac{dT}{dt}\right) = \alpha(T_{eq} - T), \tag{2}$$

where α is a constant that depends on sample geometry, insulation, etc. In general, when microwave power is applied to a sample that was not in thermal equilibrium with its surroundings at the start of exposure:

$$\frac{dT}{dt} = \frac{S}{C} + \alpha(T_{eq} - T) \tag{3}$$

The solution to eqn. (3) can be written in the form

$$T - T_o = \left(\frac{S}{\alpha C} + T_{eq} - T_o\right)(1 - e^{-\alpha t}) \tag{4}$$

where T_o is the control temperature at t = 0. Either eqn. (3) or eqn. (4) can be used to determine the SAR. From eqn. (3),

$$\left(\frac{dT}{dt}\right)_S - \left(\frac{dT}{dt}\right)_{S=0} = \frac{S}{C}, \tag{5}$$

where The subscript S indicates the application of microwave power corresponding to SAR S. Therefore, determination of the rate of temperature change before and during application of microwave power, or during and after the application of microwave power, determines the SAR.

If the sample is in thermal equilibrium before exposure begins, eqn. (4) reduces to the simpler form

$$T - T_o = \frac{S}{\alpha C}(1 - e^{-\alpha t}) \tag{6}$$

which can be used to find S from T(t). In general, the approach using eqn. (5) is preferable, since for times near t = 0, the exponential can be expanded in quadratic form.

In practice, this analysis is performed automatically. At the end of a run, the point of inflection, or turning point, in T(t) is found numerically. This is done by checking the change in slope of the temperature as a function of time. In practice, this change in slope occurs immediately upon the application or removal of microwave power, within the time resolution of the data acquisition system. The temperature profile to the left and right

of this point is fitted by least-squares to a linear or quadratic function and the change in slope at the beginning of the exposure period yields the SAR.

In practice temperatures in the exposed sample are recorded each 1, 2 or 3 seconds, the readings from the sham-exposed sample being taken at 10 times longer intervals. In experiments in which the sample is microwave heated for longer periods at a predetermined elevated temperature, measurements are made less frequently once equilibrium is reached, and serve only as control of the stability of exposure conditions.

Measurements made in air or water in the incubator outside the waveguides, demonstrated oscillations of $\pm 0.2°C$ resulting from the on-off cycle of heating controlled by the thermostat. Over a 24 hour period such oscillations may reach $\pm 0.5°C$. Measurements in medium in chamber-slides placed within the waveguides demonstrated that these oscillations are attenuated, the metal structure of the waveguide acting as a heat sink or source. Over a 24 hour period the oscillations do not exceed 0.05°C, and usually remain within 0.02°C. The sensitivity of the temperature probes is nominally 0.01°C, however comparison of successive readings permits extrapolation to 0.005°C. Based on data in the literature the biological endpoints examined are not affected by temperature excursions within 0.5°C.

The software written for reading temperatures from two Narda probes and for "smoothing and plotting of experimental data", *i.e.*, for SAR computation is enclosed as attachement 1. The software was slightly modified to accommodate the alternate possibility for taking reading from Vitek probes.

The dosimetric system described above can be used in experiments on the effects of heating caused not only by microwaves but also by any means (eg. conventional, ultrasound). Because of its wide applicability the system is the subject of two brief communications: one at the IRPA-7 Congress in April, 1988 in Sydney Australia (attachment 2), and a second one at the 10th Annual Meeting of the BEMS in June, 1988 in Stamford, CT (attachment 3). Finally, a detailed description was published in Health Physics 56, 303-307, 1989, (attachment 4).

STUDIES OF LYMPHOCYTE CULTURES

Collection of Blood Samples and Separation of Lymphocytes

Approval for the use of human blood was obtained November 19, 1986 from the Human Subjects Research Review Board, Office of the Surgeon General, Department of the Army. Volunteers were informed about the nature of the study, potential risks and the potential benefit derived from the study, and signed the informed consent form, as approved by the HSRRR. 20ml of blood was withdrawn by venipuncture under aseptic conditions into two heparinized (143 USP units) sterile evacuated blood collection tubes (Vacutainer No. 6480, Becton and Dickinson). The blood was transported to the laboratory at room temperature. Within one hour from withdrawal mononuclear cells (lymphocytes and monocytes) were separated under sterile conditions from the whole blood by gradient centrifugation in Lymphocyte Separation Medium (Boehringer, Mannheim, West Germany) according to the manufacturer's instructions. Following separation the cell suspension was washed twice in RPMI 1640 cell culture medium (Gibco). An aliquot was withdrawn for the determination of the cell concentration, cell viability and cytological (cytocentrifuge) preparations, the remainder was used for establishing cultures.

The cells were counted in a bright line hemacytometer (Neubauer type, Spencer). Viability was tested by the Trypan blue exclusion test, neutral red stain for the vacuome, and Janus green stain for mitochondria. Cytocentrifuge (Cytospin 2 cytocentrifuge, Shandon) preparations were air dried, fixed in methanol, stained by a combined Wright-Giemsa stain and cell morphology was analyzed under a microscope (see below).

The usual yield of cells ranged from 1×10^7 to 3×10^7. The suspension consisted of 90-95% lymphocytes, the remainder of cells being monocytes and occasional (below 1%) granulocytes. The viability of cells ranged from 96.5 to 99.5%, and was usually about 98%.

Cell Cultures

Four-chamber culture chamber slides were filled under sterile conditions with 1 ml of chromosome medium 1A (Gibco) with or without phytohemagglutinin (PHA) depending on the protocol appropriate for the particular experiment. 10^6 cells were added to 3 of the chambers, the 4th contained medium only. A 0.2 mm hole was drilled over the chamber

that does not contain cells for the introduction of the temperature probe. (For experiments on spontaneous transformation a set of experimental samples consists of four slides: one with PHA (5 or 10 mg/ml) positive control incubated in a conventional incubator, and three without PHA. Out of these one is incubated in a conventional incubator at 37°C. one is microwave-exposed and one is sham exposed).

Cell Harvest

Following incubation the cultures without PHA were mixed within the culture chamber using a Pasteur pipette, and transferred to microfuge tubes. The cell count was established, viability was tested and cytocentrifuge preparation were made (as described above).

In the case of cultures incubated with PHA, 10μl of colcemide solution (10 mg/ml, Gibco) were added to each chamber 2 hours before harvest. At harvest the cultures were transferred to microfuge tubes, spun down at 10^3 rpm for 8 min in an Eppendorf micro centrifuge, and the supernatant was discarded. 1 ml 1% sodium cirtrate solution prewarmed to 37°C was added with vortexing. The cells were kept in this solution for 8 min, spun down under the same conditions as above, and the supernatant was discarded. Fixative (cold glacial acetic acid: methanol, 1:3 vol/vol) was added dropwise with vortexing. After at least 20 min the fixative was changed once or twice. A droplet of the cell solution in fixative was placed on a microscope slide wet with 20% ethanol, and the slides were flamed. Following this the slides were air dried, and stained with Giemsa solution in phosphate buffer pH 6.8 for 8 min or treated with trypsin and stained with Giemsa according to the Seabright procedure (G-banding).

Analysis of Microscope Preparations

Cytocentrifuge slides were examined under a 100× oil immersion objective and the cell and nucleus areas were determined using an "Optomax" image analyzer. The original software supplied by the manufacturer was rewritten. The slides was coded and the person who made the measurements was not aware of the treatment (exposed, sham-exposed or conventional incubator). The code number of the slide, the area of the cell, the area of the nucleus, the nucleus-cytoplasmic ratio and a symbol for morphological features of the cell

(S for a small lymphocyte, 1 for an intermediate form and B for a lymphoblastoid cell) were recorded, and stored on a floppy disk.

The process of lymphoblastoid transformation was initially evaluated by most researchers on the basis of cell morphology. Following stimulation the lymphocyte undergoes gradual enlargement, accompanied by structural alterations of the nuclear chromatin, and becomes an intermediate cell. The transition of the intermediate into a lymphoblastoid cell consists in further enlargement of the cell, accompanied by additional changes in chromatin structure and the appearance of nuclei. Following a phase of DNA synthesis (S-phase) and pre-mitotic period during which no morphological changes occur (g_2 phase), the lymphoblastoids undergo cell division. Evaluation of the transformation process based on qualitative assessment of cell morphology is subjective, and varies with different observers. Because of this, determination of tritiated thymidine incorporation into DNA is the most widely used method for a quantitative assessment of lymphoblastoid transformation. However, this method does not fully reflect the process of lymphoblastoid transformation, being only a measure of the rate of DNA synthesis. Small and intermediate lymphocytes, as well as lymphoblastoids in other stages of the cell cycle rather than the S-phase are not taken into account. However, using this method it is not possible to distinguish between an increase or a decrease in tritiated thymidine incorporation caused by a change in the number of cells synthesizing DNA or by a change in the rate of synthesis alone. As the present study requires incubation of cells at elevated temperatures, which may affect not only the basal metabolic rate but also the DNA synthesis rate, a morphological approach supplemented by objective measurements of the cell and nucleus area was adopted.

To validate this approach, three different observers analyzed cytocentrifuge preparations from cultures incubated with PHA at three concentrations of 5, 7.5, and 10 mg/ml. The last one was indicated by the manufacturer as the optimal concentration. The distribution of the size (area) of the cells demonstrated a dependence on PHA concentration. The area of the nucleus was distributed randomly and did not correlate with the morphological classification of the cell. Consequently the nucleus-cytoplasmic ratio could not be used for the evaluation of the transformation process.

Cells of size between 80 and 120 μm^2 were classified by different observers as small or intermediate lymphocytes, and cells between 190 and 210 μm^2 as intermediate or blastoid forms. Therefore based on morphology and measurements of the cell area, cells up to $100\mu m^2$ were grouped in one class of small lymphocytes. Cells larger than $200\mu m^2$ were considered as blastoid forms. Intermediate lymphocytes were additionally subdivided into three groups: larger than $100\mu m^2$ and smaller or equal to $120\mu m^2$, larger than $120\mu m^2$ and smaller or equal to $150\mu m^2$, and larger than $150\mu m^2$ and smaller or equal to $200\mu m^2$.

The distribution of the cell size in preparations from cultures incubated 72h with PHA at the three tested concentration is shown in Table 1. Significant differences were found in the number of small lymphocytes (area $\leq 100\mu m^2$) and lymphoblastoids (area $> 200\mu m^2$). The decrease in the number of small lymphocytes and the increase in the number of blastoid cells with increasing PHA concentration validates the use of measurements of the cell area for the assessment of lymphocyte transformation.

Table 1

The Distribution of cell size (area, μm^2) following 72h incubation with PHA at Various Concentrations (mg/ml)

PHA mg/ml Area (a) μm^2	5.0	7.5	10.0
$a \leq 100$	42.5 ± 3.7	26.8 ± 4.5	17.4 ± 2.5
$100 < a \leq 120$	13.4 ± 2.0	13.0 ± 2.8	5.3 ± 1.1
$120 < a \leq 150$	15.6 ± 3.4	12.1 ± 3.1	10.0 ± 2.1
$150 < a \leq 200$	19.0 ± 2.6	24.3 ± 2.8	22.6 ± 3.0
$a > 200$	10.6 ± 4.8	25 ± 3.5	46.2 ± 4.5

Chromosomal preparations were examined using a 100× oil immersion objective. Metaphase plates were analyzed for numerical structural aberrations.

Results were analyzed using the "Statgraphics" program by Statistical Graphics Corporation. Analysis of variance (ANOVA) was used to compare the numbers of cells in particular classes, distributions were compared using multiple regression methods. Results were considered significant at the $p \leq 0.05$ level.

Effects Of Exposure On Spontaneous Transformation

Blood was obtained from healthy donors by venipuncture, lymphocytes were separated as described above, and transferred to Chromosome Medium 1A without PHA (Gibco). The cell concentration was adjusted to 10^6/ml. One milliliter of cell suspension was introduced into each of the 3 chambers of the chamber-slide, the 4th chamber contained medium only, and contained the sensor for the dosimetric system. Thus each experiment was carried out in triplicate, and usually repeated 3 times (a total of 9 samples), some experiments were carried out twice (6 samples).

Each experiment comprised three chamber-slides, one exposed, the second one sham exposed, and the third one incubated in a separate incubator set at 37°C, serving as control.

The experiments were carried out in three series:

• exposed to CW 2450 MHz at a non-heating level, *i.e.* at an average SAR of about 1 W/kg (ranging from 0.8 to 1.3 W/kg), and at heating levels resulting in an increase in temperature of the sample by 0.5°C (SAR 1.8-2.3 W/kg), 1°C (SAR 3.5-4.5 W/kg), 1.5°C (SAR 6.8-8.3 W/kg), and 2°C (9.8-12.3 W/kg); the temperature of the sample varied over the incubation period by ±0.2°C; the range of SARs is indicated taking into account the experimentally observed variations and the ±10% non-uniformity of energy absorption over the three chambers of the chamber slide;

• exposed to PW 2450 MHz at the same non-heating and heating levels of temperature and SARs (for these PW exposures the microwave pulse width was 1μs and the pulse repetition frequency varied from 100 to 1000 pps as the exposure changed from non-heating to the highest heating level);

• incubated at the temperatures obtained by microwave heating (37.5°C, 38°C, 38.5°C, and 39°C) in an incubator with adjusted thermostat, samples incubated at 30°C serving as controls and comparison to the non-heating microwave level.

There were no significant differences between sham-exposed and control cultures, and therefore the results from these were pooled. Table 2 presents the distribution of cell sizes in these cultures.

Table 2

The distribution of cell sizes in control and sham-exposed cultures incubated for 120h

Area(a) μm^2	Number of cells mean±s.d.
$a \leq 100$	53.7 ± 2.9
$100 < a \leq 120$	30.9 ± 1.8
$120 < a \leq 150$	10.1 ± 1.0
$150 < a \leq 200$	2.1 ± 0.8
$a > 200$	1.2 ± 0.9

Table 3 presents the distribution of cell sizes in conventionally heated cultures. There are no significant differences between cultures incubated at 30°C and 37.5oC. At 38°C the number of small lymphocytes (area< $100\mu m^2$decreases, and the number of cells with areas between $120\mu m^2$and $200\mu m^2$increases, thus indicating an enhancement of the transfomation process. At 38.5°C the number of cells with an area between 100 and $120\mu m^2$decreases, and the number of cells with area between 150 and $200\mu m^2$increases. No blastoid cells were found. This may be interpreted as enhancement of transformation (blastoid cells divided and the daughter cells are smaller) or an inhibition. The latter interpretation is less likely because of the results obtained at 38°C , and the lack of cell destruction in the preparations.

Table 3. The distribution of cell sizes in control cultures incubated at 37.5, 38, 38.5, and 39°C , with conventional heating

			Number of cells mean± s.d.		
Incubation temperature Area (a) μm^2	37°C	37.5°C	38°C	38.5°C	39°C
a≤100	55.2±3.8	54.8±2.2	39.6±3.0	53.0±4.5	88.7±2.2
100< a ≤120	32.4±2.9	29.4±3.6	29.6±7.1	14.7±2.3	9.1±1.9
120< a ≤150	8.8±2.0	11.2±1.7	23.2±3.4	16.2±2.2	1.6±1.4
150< a ≤200	1.6±0.9	1.3±1.0	3.4±1.0	15.8±2.0	0.3±0.5
a >200	1.0±0.9	2.4±1.7	4.4±1.0	0	0.1±0.3

In all experiments described above the total count of cells did not change significantly, being of the order of 10^6/ml with variations within the error of the method. Cell viability also did not change, remaining at the initial level of 94 to 98%. At 39°C the number of small lymphocytes increased significantly, while the number of lymphoblastoid cells was smaller than in cultures incubated at 37, 37.5, and 38°C , the difference, however, was not statistically significant. Cellular debris was present in the preparations. The total cell count decreased from 6.7×10^5 to 9.1×10^5 cells/ml. Viability of cells decreased, and ranged from 84 to 93%. These results may be interpreted as indicative of heat cell killing.

Table 4 presents the results obtained following CW 2450 MHz microwave heating. No statistically significant differences were noted between this and the preceding series, with the possible exception of cultures heated to 39°C . At this temperature signs of cell destruction were noted. The total cell count ranged from 6.4×10^5 to 8.5×10^5 cells/ml. Viability varied from 75.5 to 81.5%. In view of this, no reliable determination of the distribution of cell sizes could be made.

Table 4. The distribution of cell sizes in cultures exposed to CW 2450 MHz microwaves at non-heating (37°C) and heating levels

			Number of cells mean ± s.d		
Incubation temperature Area (a) μm^2	37°C	37.5°C	38°C	38.5°C	39°C
a≤100	53.3±2.1	40.0±3.4	39.4±3.0	43.6±4.3	partial cell
100< a ≤120	31.7±3.0	36.0±1.9	30.0±4.9	15.2±2.7	destruction,
120< a ≤150	10.2±2.4	12.2±2.0	22.8±2.8	17.6±1.7	measurements
150< a ≤200	1.7±0.7	4.8±1.6	2.1±0.9	22.6±2.8	unreliable
a >200	1.0±0.9	2.4±1.7	5.7±1.7	1.0±0.9	

Table 5 presents the results obtained following PW 2450 MHz microwave heating. Exposure at the non-heating level results in an increase in the number of intermediate forms with an area greater than 150$\mu m^2$and smaller or equal to 200μm^2, the number of blastoid forms increases too. These increases may be considered real in view of the results obtained at 37.5 and 38°C . At these temperatures a statistically significant increase in the numbers of lymphoblastoid cells (area≤200μm^2) occurs. At 38.5°C cell destruction renders the determination of cell size unreliable, and no measurements were made. In view of this, planned experiments at 39°C were not performed, no reliable results were expected.

Table 5. The distribution of cell sizes in cultures exposed to PW 2450 MHz microwaves at non-heating (37°C) and heating levels

			Number of cells mean ± s.d		
Incubation temperature Area (a) μm^2	37°C	37.5°C	38°C	38.5°C	39°C
a≤100	59.7±3.9	44.6±3.8	46.8±4.7	partial	
100< a ≤120	15.4±1.8	20.6±2.4	16.3±2.0	cell	
120< a ≤150	13.7±1.4	16.6±1.5	16.3±2.0	destruction	not
150< a ≤200	4.9±1.8	3.9±1.0	10.6±2.0	measurements	performed
a >200	6.3±1.7	14.4±1.2	15.9±3.7	unreliable	

Effects of Exposure on PHA-induced Transformation

Blood was obtained from healthy donors by venipuncture, lymphocytes were separated as described above, and transferred to Chromosome Medium 1A containing 5 mg/ml PHA (Gibco). The cell concentration was adjusted to 10^6 cells/ml. One milliliter of cell suspension was introduced into each of 3 chambers of the chamber slide, the 4th chamber contained medium only, and housed the sensor of the dosimetric system. Thus each experiment was performed in triplicate, and usually was repeated three times: in some instances twice. Each experiment comprised three chamber-slides, one exposed, a second one sham-exposed, and a third one incubated at 30°C in a separate incubator, serving as control. The conditions chosen for these experiments were based on previously obtained results. No experiments were carried out under conditions that did not demonstrate significant differences between experimental and control samples, or resulted in cell destruction rendering measurements unreliable. The experiments were carried out in three series:

• exposed to CW 2450 MHz microwaves over the whole 72h incubation period at a non-heating level, *i.e.* at an average SAR of about 1W/kg (ranging from 0.7 to 1.3 W/kg) and at a level resulting in an increase in the temperature of the sample by 1°C (38°C) *i.e.* at an average SAR of about 4W/kg; the temperature of the sample varied over the course of the experiment by ± 0.2°C ; the indicated range of SAR takes into account the

experimentally observed variations in different chambers of the chamber-slide;

• exposed over the whole 72h of the incubation period to PW 2450 MHz microwaves at the same levels of average SAR and temperature as in the preceding series; an additional set of experiments consisted in exposure to PW 2450 MHz at an average SAR resulting in an increase in the temperature of the sample by 1°C over the first, second and third 24h of the 72h incubation period, following which the cultures were harvested.

• cultures incubated for 72h in an incubator adjusted to 38°C and compared to cultures incubated at 37°C .

The results of these experiments are summarized in Table 6.

Table 6. The distribution of cell sizes following 72h exposure to PW 2450 MHz microwaves at a non-heating (37°C) and heating level (38°C) compared to sham-exposed samples.

		Number of cells mean ± s.d	
Incubation temperature Area (a) μm^2	Shams (37°C)	37°C	38°C
a≤100	43.2±7.4	14.3±5.1	11.7±5.3
100< a ≤120	14.3±4.6	16.7±3.0	5.9±2.9
120< a ≤150	14.9±5.3	20.0±6.2	7.8±2.7
150< a ≤200	11.7±5.1	19.9±4.6	14.5±4.1
a >200	15.9±6.4	29.1±6.6	57.7±10.4

Control and sham-exposed cultures did not differ significantly from each other. Heating to 38°C by conventional means and by CW microwave exposure enhanced transformation to a limited extent. Exposure to CW microwaves at a non-heating level did not affect the transformation process. Exposure to PW 2450 MHz during the whole incubation period of 72h enhanced transformation at both the heating and non-heating levels. Exposure to PW 2450 MHz at the heating (38°C) level during the first 24h of the incubation period did not affect transformation, no significant differences between exposed and sham-exposed samples were demonstrated. Exposure during the second 24h of the incubation

period resulted in an inhibition of transformation and a complete absence of cell divisions. Exposure during the last 24h enhanced transformation.

No chromosomal aberrations were demonstrated under any exposure conditions.

CONCLUSIONS

Spontaneous transformation of human lymphocytes *in vitro* is enhanced by conventional heating and CW 2450 MHz microwave heating. No significant differences were demonstrated between preparations obtained from cultures heated to the same temperature by conventional means and by CW microwaves. PW microwave exposure enhances transformation at a non-heating level. Further enhancement of transformation was seen in preparations heated by PW exposure to 37.5 and 38°C , the number of transforming cells being significantly higher than in cultures heated by CW exposure. Following PW exposure at the heating level resulting in an increase in the termperature of the sample by 1.5°C (to 38.5°C) or 2°C (to 39°C) numerous cells undergo destruction making the interpretation of the preparation unreliable.

Effects of CW exposure seem to be related to heating, *i.e.* depend upon the increase in temperature. Effects obtained following PW exposure differ from those seen following CW exposure, PW exposure enhances transformation at non-heating levels, while CW exposure does not. Thus effects of CW and PW 2450 MHz microwaves are different and are not related to the average SAR.

PHA-induced transformation is affected by exposure in a similar maner, and leads to the same conclusions. Additionally this part of the study demonstrated the dependence of the effects on the stage of the transformation process. Exposure during initial stages (first 24h of the 72h incubation period) is without effect. Exposure during the second 24h of the 72h incubation period blocks cell division and decreases transformation, while exposure during the last 24h enhances transformation.

Results of this study were presented at the 10th Annual Meeting of the Bioelectromagnetics Society (2 presentations) in Stamford, Connecticut in 1988 and at the 7th International Congress of the International Radiation Protection Association (IRPA) in Sydney, Australia (2 presentations). Copies of abstracts are included in this report. One

paper describing the exposure system and dosimetry protocols has already been published in Health Physics (copy included). Two further manuscripts are in preparation and will be submitted later. Also included as an attachment to this report is the computer software used for experimental monitoring, temperature history data acquisition, and dosimetry.

Attachment #1

PROGRAM FOR COLLECTING DATA

```
10 OPTION BASE 1
20 DISP "ARE YOU USING VITEK OR NARDA PROBES,TYPE V OR N"
30 INPUT PRBTYP$
40  PROBC1=9.6184 @ PROBC2=41.459 ! Calibration factors for Narda probe 1
50  PROBC3=9.9121 @ PROBC4=42.386 ! Calibration factors for Narda probe 2
60  IF PRBTYP$="N" THEN GOTO 90 ! Probe type Narda - don't reset calib. factors
70  PROBC1=100 @ PROBC2=0 ! Calibration factors for Vitek probe
80  PROBC3=100 @ PROBC4=0 ! Calibration factors for Vitek probe
90 ON KEY# 1,"POWER ON" GOSUB 1380
100 ON KEY# 4,"NEW OBS INT" GOTO 1100
110 ON KEY# 7,"POWER OFF" GOSUB 1390
120 MASS STORAGE IS ":D500"
130 DIM ATEMP(1000),ATIME(1000),BTEMP(1000),BTIME(1000),PONT(50),POFFT(50)
140 DIM ANUM(20),BNUM(20),IPON(20),IPOFF(20)
150 CLEAR
160 ! BEEP 40,100 @ WAIT 50 @ BEEP 40,100 @ WAIT 50 @ BEEP 40,100 @ WAIT 50 @ BE
EP 80,150
170 DISP "ENTER DAY,MONTH,YEAR"
180 INPUT EXPDATE$
190 EXPDATE=0
200 DISP "ENTER SAMPLE IDENTIFIER"
210 INPUT SAMPLE$
220 EXPDATA$=EXPDATE$&SAMPLE$
230 DISP "ENTER TIME OF DAY - 24 HOUR CLOCK" @ INPUT TOD$
240 ON ERROR GOTO 300
250 CREATE EXPDATA$&":D501",1,32000
260 ASSIGN# 1 TO  EXPDATA$&":D501"
270 ! PRINT# 1 ; EXPDATE$
280 ! PRINT# 1 ; SAMPLE$
290 GOTO 390
300 IF ERRN =128 THEN DISP "DISC IS FULL - INSERT A NEW DISC AND START AGAIN"
310 IF ERRN =128 THEN GOTO 340
320 IF ERRN =63 THEN GOTO 350
330 DISP "AN ERROR HAS OCCURRED - PRESS CONTINUE TO START AGAIN"
340 PAUSE @ GOTO 150
350 DISP "THIS DATA FILE NAME ALREADY EXISTS - ENTER ANOTHER SAMPLE NUMBER"
360 INPUT SAMPLE$
370 EXPDATA$=EXPDATE$&SAMPLE$
380 GOTO 250
390 KK=0 ! Counter for new observation times for probes A and B.
400 BIN=0 ! Holds previous number of observations from probe A.
410 NDPTS=0 ! Counter for number of data points recorded.
420 CLEAR
430 DISP "This program will store 1000 temps. and times for both probes."
440 DISP "ENTER TOTAL OBSERVATION TIME IN SECONDS."
450 INPUT OBSTIME
460 DISP "Probe A should be in the exposed/experimental culture."
470 DISP
480 DISP "ENTER OBSERVATION INTERVAL FOR PROBE A IN SECONDS."
490 INPUT NA
500 IF NA>OBSTIME THEN 520
510 GOTO 540
520 DISP "You selected an obs. interval > the total obs. time; try again."
530 GOTO 430
540 AJOBS=INT (OBSTIME/NA)
550 NA=NA*1000
560 HOLD=AJOBS+BIN
570 IF HOLD>1000 THEN 590
580 GOTO 610
590 DISP "You have requested too many readings of probe A; try again."
600 GOTO 430
```

```
610 DISP "The observation interval for probe B should be an integer multiple"
620 DISP "of, and ideally < or = 10 times that for probe A."
630 DISP
640 DISP "ENTER OBSERVATION INTERVAL FOR PROBE B IN SECONDS."
650 INPUT NB
660 BJOBS=INT (OBSTIME/NB)
670 NB=NB*1000
680 IF FP (NB/NA)#0 THEN 700
690 GOTO 720
700 DISP "The obs. time for  B is not a multiple of that for A; try again."
710 GOTO 640
720 REMOTE 502 ! DVM to remote.
730 CLEAR @ KEY LABEL
740 REMOTE 507 ! Relay to remote.
750 LOCAL LOCKOUT 5 ! Local lockout for DVM and relay.
760 OUTPUT 502 ;"FOR2TOX" ! DVM to DCV,2V range, and cont. on talk.
770 OUTPUT 507 "A12" ! Connect all A1&A2 terminals to C1&C2 terminals.
780 ENTER 502 ; A$ ! Trial read of DVM.
790 IJ1=0 ! Set counter for number of times power turned on.
800 IJ2=0 ! Set counter for number of times power turned off.
810 J=1 ! Set counter for reading probe B.
820 NT=NB/NA ! Set interval between reading probe B.
830 IF KK>0 THEN 860
840 SETTIME 0,0 ! Zero clock for easy interpretation only.
850 ! Loop. read probe A, read time, put values into arrays.
860 NNA=1 @ NNB=1
870 FOR I=1 TO AJOBS @ ENTER 502 ; A$@ ATIME(I)=TIME +(DATE -EXPDATE)*86400 @ A
EMP(I)=VAL (A$[5])
880 ATEMP(I)=PROBC1*ATEMP(I)+PROBC2 ! Evaluate temp. from voltage reading.
890 NNA=NNA+1
900 DISP "ATEMP=",ATEMP(I),"TIME=",ATIME(I)
910 IF I=J*NT THEN 930 ! Check to see if probe B is to be read.
920 GOTO 1010
930 OUTPUT 507 "B1" ! Switch  B1 to  C1 prior to reading probe B.
940 WAIT 200 ! Wait 200ms for DVM to read probe B.
950 ! Read probe B, read time, put values into arrays. Switch A1 to C1.
960 ENTER 502 ; B$@ BTIME(J)=TIME +(DATE -EXPDATE)*86400 @ BTEMP(J)=VAL (B$[5])
@ OUTPUT 507 "A1"
970 BTEMP(J)=PROBC3*BTEMP(J)+PROBC4 ! Evaluate temp. from voltage reading.
980 NNB=NNB+1
990 DISP "BTEMP=",BTEMP(J),"TIME=",BTIME(J) @ DISP @ DISP @ DISP @ KEY LABEL
1000 J=J+1 ! Increment counter on read probe B.
1010 WAIT NA ! Wait time between reading probe A.
1020 NEXT I ! Increment loop.
1030 LOCAL 5
1040 OUTPUT 702 "B12" ! Simply turn off lights on relay.
1050 DISP "Storing data on disc. You will be prompted for more inputs shortly."
1060 DISP @ DISP @ DISP
1070 WAIT 2000
1080 GOSUB DISCPRINT
1090 GOTO 1400
1100 CLEAR
1110 DISP "Do not press any keys until the computer responds with a beep and"
1120 DISP "displays 'Data storage is complete'. You will then be prompted to"
1130 DISP "key in new observation data."
1140 WAIT 5000
1150 GOSUB DISCPRINT
1160 GOTO 440
1170 DISCPRINT: ! Write all data to disc for this observation interval.
1180 PRINT# 1 ; NNA-1
1190 FOR I=1 TO NNA-1
1200 PRINT# 1 ; ATEMP(I),ATIME(I)
```

```
1210 NEXT I
1220 PRINT# 1 ; NNB-1
1230 FOR I=1 TO NNB-1
1240 PRINT# 1 ; BTEMP(I),BTIME(I)
1250 NEXT I
1260 PRINT# 1 ; IJ1 ! The number of times power turned on.
1270 IF IJ1=0 THEN 1290
1280 FOR I=1 TO IJ1 @ PRINT# 1 ; PONT(I) @ NEXT I
1290 PRINT# 1 ; IJ2 ! The number of times power turned off.
1300 IF IJ2=0 THEN 1320
1310 FOR I=1 TO IJ2 @ PRINT# 1 ; POFFT(I) @ NEXT I
1320 PRINT# 1 ; TOD$ ! Writes time of day to disc
1330 KK=KK+1
1340 BIN=AJOBS
1350 NDPTS=NDPTS+2*(NNA+NNB)+IJ1+IJ2+10
1360 BEEP @ CLEAR @ DISP "Data storage is complete." @ RETURN
1370 GOTO 1400
1380 BEEP @ IJ1=IJ1+1 @ DISP "Power on" @ PONT(IJ1)=TIME @ RETURN
1390 BEEP @ IJ2=IJ2+1 @ DISP "Power off" @ POFFT(IJ2)=TIME @ RETURN
1400 DISP "Do you wish to record more data? ENTER (Y)ES or (N)O."
1410 INPUT REPLY$
1420 IF REPLY$="Y" THEN 420
1430 ASSIGN# 1 TO *
1440 DISP "The final disc management is now being completed"
1450 ASSIGN# 1 TO EXPDATA$&":D501"
1460 IA1=1 @ IATOT=0 @ IB1=1 @ IBTOT=0 @ ION1=1 @ IONT=0 @ IOFF1=1 @ IOFFT=0
1470 FOR J1=1 TO KK
1480 READ# 1 ; ANUM(J1)@ IATOT=IATOT+ANUM(J1)
1490 FOR I=IA1 TO IATOT @ READ# 1 ; ATEMP(I),ATIME(I)@ NEXT I
1500 READ# 1 ; BNUM(J1)@ IBTOT=IBTOT+BNUM(J1)
1510 FOR I=IB1 TO IBTOT @ READ# 1 ; BTEMP(I),BTIME(I)@ NEXT I
1520 READ# 1 ; IPON(J1)@ ION1=ION1+IPON(J1) @ IF IPON(J1)=0 THEN 1540
1530 FOR I=ION1 TO ION1 @ READ# 1 ; PONT(I)@ NEXT I
1540 READ# 1 ; IPOFF(J1)@ IOFFT=IOFFT+IPOFF(J1) @ IF IPOFF(J1)=0 THEN 1560
1550 FOR I=IOFF1 TO IOFFT @ READ# 1 ; POFFT(I)@ NEXT I
1560 IA1=IA1+IATOT @ IB1=IB1+IBTOT @ ION1=ION1+IONT @ IOFF1=IOFF1+IOFFT
1570 NEXT J1
1580 READ# 1 ; TOD$
1590 ASSIGN# 1 TO *
1600 PURGE EXPDATA$&":D501"
1610 PACK ":D501"
1620 NDPTS=8+NDPTS
1630 CREATE EXPDATA$&":D501",1,NDPTS
1640 ASSIGN# 1 TO EXPDATA$&":D501"
1650 PRINT# 1 ; IATOT @ DISP "TOTAL PROBE A READINGS = ",IATOT
1660 FOR I=1 TO IATOT @ PRINT# 1 ; ATEMP(I),ATIME(I) @ NEXT I
1670 PRINT# 1 ; IBTOT @ DISP "TOTAL PROBE B READINGS = ",IBTOT
1680 FOR I=1 TO IBTOT @ PRINT# 1 ; BTEMP(I),BTIME(I) @ NEXT I
1690 PRINT# 1 ; IONT @ DISP "MW POWER TURNED ON ",IONT," TIMES" @ FOR I=1 TO ION
T @ PRINT# 1 ; PONT(I) @ DISP PONT(I) @ NEXT I
1700 PRINT# 1 ; IOFFT @ DISP "MW POWER TURNED OFF ",IOFFT," TIMES" @ FOR I=1 TO
IOFFT @ PRINT# 1 ; POFFT(I) @ DISP POFFT(I) @ NEXT I
1710 PRINT# 1 ; TOD$ @ DISP "Time of day",TOD$
1720 ASSIGN# 1 TO *
1730 DISP "Sample number is ",SAMPLE$
1740 DISP "Date of exposure is ",EXPDATE$
1750 DISP "Finished the disc management. GOODBYE, HAVE A NICE DAY!"
1760 END
```

PROGRAM FOR DATA ANALYSIS, CURVE SMOOTHING AND GRAPHICS _ version A

```
10 ! ******************************************************
20 ! *                                                    *
30 ! * PROGRAM TO READ TEMPERATURE READINGS TAKEN FROM    *
40 ! *  TWO NARDA PROBES AND STORED ON DISC, PLOT DATA    *
50 ! * IN VARIOUS FORMS AND DO DOSIMETRY CURVE FITTING    *
60 ! *                                                    *
70 ! * WRITTEN BY CHRISTOPHER C DAVIS, DECEMBER. 1986     *
80 ! *                                                    *
90 ! ******************************************************
100 OPTION BASE 1
110 MASS STORAGE IS ":D500"
120 DIM ATEMP(1000),ATIME(1000),BTEMP(1000),BTIME(1000),PONT(50),POFFT(50)
130 DIM ANUM(20),BNUM(20),IPON(20),IPOFF(20),DUMMY$[50]
140 ! *** FUNCTION TO GIVE INVERSE VIDEO DISPLAYS
150 DEF FNIVID$(DUMMY$)
160 LENGTH=LEN (DUMMY$)
170 FOR II=1 TO LENGTH
180 DUMMY$[II,II]=CHR$ (NUM (DUMMY$[II,II])+128)
190 FNIVID$=DUMMY$
200 NEXT II
210 FN END
220 CLEAR
230 DISP FNIVID$("DATA DATE?")
240 DISP FNIVID$("DATE FORMAT")
250 DISP FNIVID$("DAY MONTH,YEAR")
260 INPUT EXPDATE$
270 DISP FNIVID$("ENTER SAMPLE ID")
280 INPUT SAMPLE$
290 EXPDATA$=EXPDATE$&SAMPLE$
300 ON ERROR GOTO 320
310 GOTO 370
320 IF ERRN =67 THEN DISP "YOU HAVE ENTERED THE WRONG FILE NAME"
330 IF ERRN #67 THEN DISP "AN ERROR HAS OCCURRED - START AGAIN"
340 IF ERRN #67 THEN GOTO 10
350 CAT ":D501"
360 DISP "THESE ARE THE DATA FILES - CHOOSE THE ONE YOU WANT" @ GOTO 230
370 ASSIGN# 1 TO  EXPDATA$&":D501"
380 NUMSMO=0 @ NTEST=0 @ SAR=0 @ TOD$="0"
390 IATOT=0 @ IBTOT=0 @ ION1=0 @ IOFFT=0 @ IA1=1 @ IB1=1 @ ION1=1 @ IOFF1=1
400 READ# 1 ; IATOT ! READ NO OF PROBE A READINGS
410 FOR I=IA1 TO IATOT @ READ# 1 ; ATEMP(I),ATIME(I)@ NEXT I ! PROBE A DATA
420 READ# 1 ; IBTOT ! READ NO OF PROBE B READINGS
430 FOR I=IB1 TO IBTOT @ READ# 1 ; BTEMP(I),BTIME(I)@ NEXT I ! PROBE B DATA
440 READ# 1 ; ION1 ! NO OF TIMES MW PWR TURNED ON
450 FOR I=ION1 TO ION1 @ READ# 1 ; PONT(I)@ NEXT I ! ON TIMES
460 READ# 1 ; IOFFT ! NO OF TIMES MW PWR TURNED OFF
470 FOR I=IOFF1 TO IOFFT @ READ# 1 ; POFFT(I)@ NEXT I ! OFF TIMES
480 IA1=IA1+IATOT @ IB1=IB1+IBTOT @ ION1=ION1+ION1 @ IOFF1=IOFF1+IOFFT
490 ON ERROR GOTO 499
491 READ# 1 ; TOD$
499 OFF ERROR @ ASSIGN# 1 TO  *
500 ! PURGE EXPDATA$&":D501"
510  DISP "TOTAL PROBE A READINGS = ",IATOT
520 ! FOR I=1 TO IATOT @ DISP ATEMP(I),ATIME(I),I @ NEXT I
530  DISP "TOTAL PROBE B READINGS = ",IBTOT
540 ! FOR I=1 TO IBTOT @ DISP BTEMP(I),BTIME( ),I @ NEXT I
550 DISP "MW POWER TURNED ON ",ION1," TIMES"
560 DISP "MW POWER TURNED OFF ",IOFFT," TIMES"
570 DISP "THE MW PWR WAS TURNED ON AT THE FOLLOWING TIMES"
580 FOR I=1 TO ION1 @ DISP PONT(I) @ NEXT I
590 DISP "Sample ID is ",FNIVID$(SAMPLE$)
```

```
600 DISP "Date of exposure  was ",FNIVID$(EXPDATE$)
601 IF TOD$="0" THEN GOTO 610
602 DISP "Time of day was ",FNIVID$(TOD$)
610   ! ***************************************************
620   ! *              SECTION FOR SMOOTHING              *
630   ! *     AND PLOTTING OF EXPERIMENTAL DATA           *
640   ! *                                                 *
650   ! *  WRITTEN BY Christopher C. Davis, June 1984.    *
660   ! *          MODIFIED DECEMBER 1986                 *
670   ! *                                                 *
680   ! ***************************************************
690   !
700   !
710    DIM AA(5),SN(21,21),R(20),HEAT(5),COOL(5) ! ARRAYS USED IN CURVE SMOOTHI
      G
720    NYSL$="N"
730    IF ATIME(IATOT)>BTIME(IBTOT) THEN XMAX=ATIME(IATOT) ELSE XMAX=BTIME(IBTO
      T) ! SET X AXIS MAX TO MAX OBSERVATION TIME
740    XMIN=0 ! INITIALLY SET X ORIGIN TO 0
750    JMIN=1 @ KMIN=1
760    DISP "TEMPERATURE MEASUREMENT INTERVAL WAS",XMAX
770    YMIN=10^20 @ YMAX=-(10^20)
780 ! *** FIND MAXIMA AND MINIMA OF DATA
790    FOR I=1 TO IATOT
800    IF ATEMP(I)>YMAX THEN YMAX=ATEMP(I)
810    IF ATEMP(I)<YMIN THEN YMIN=ATEMP(I)
820    NEXT I
830    FOR I=1 TO IBTOT
840    IF BTEMP(I)>YMAX THEN YMAX=BTEMP(I)
850    IF BTEMP(I)<YMIN THEN YMIN=BTEMP(I)
860    NEXT I
870    NUMPLT=0 @ IF NTEST#0 THEN CLEAR
880    DISP "DO YOU WISH TO PLOT PROBE A, PROBE B, OR BOTH? "&FNIVID$("ENTER 1,2
      OR 3")
890    DISP "YOU CAN ONLY CALCULATE SAR USING PROBE A"
900    INPUT NPROBE
910    IF NPROBE=1 THEN GOTO 930
920    GOTO 940
930    FOR I=1 TO IATOT @ BTIME(I)=ATIME(I) @ BTEMP(I)=ATEMP(I) @ NEXT I
940    IF NTEST=0 THEN GOTO 960
950    NYSL$="Y"
960    DISP "ENTER TIME REGION YOU WISH TO ANALYZE"
970    INPUT TMIN,TMAX
980    FOR I=1 TO IATOT ! FIND RANGE OF TEMP ARRAY TO BE PLOTTED
990    IF TMIN>ATIME(I) THEN JMIN=I
1000    NEXT I
1010    FOR I=1 TO IBTOT @ IF TMIN>BTIME(I) THEN KMIN=I
1020    NEXT I
1030    IF ATIME(JMIN)<BTIME(KMIN) THEN TMIN=ATIME(JMIN) ELSE TMIN=BTIME(KMIN)
1040    FOR I=1 TO IATOT @ IF TMAX>ATIME(I) THEN JMAX=I
1050    NEXT I
1060    FOR I=1 TO IBTOT @ IF TMAX>BTIME(I) THEN KMAX=I
1070    NEXT I
1080    IF ATIME(JMAX)>BTIME(KMAX) THEN TMAX=ATIME(JMAX) ELSE TMAX=BTIME(KMAX)
1090    IF NTEST=0 THEN GOTO 1400
1100    IF NYSL$="N" OR NYSL$="NO" THEN GOTO 1400
1110 ! *** SECTION FOR SELECTION OF CURVE REGION FOR SMOOTHING
1120    TEMAX=-1.E20 @ TEMIN=1.E20
1130    FOR I=JMIN TO JMAX @ IF ATEMP(I)<TEMIN THEN TEMIN=ATEMP(I)
1140    IF ATEMP(I)=TEMIN THEN JTURN=I ! Find inflection point on temp curve
1150    NEXT I
```

```
1160     DISP "NUMBER OF POINTS ON COOLING SLOPE= ",JTURN-JMIN+1
1170     DISP "NUMBER OF POINTS ON HEATING SLOPE= ",JMAX-JTURN+1
1180     DISP "IS THIS SATISFACTORY? Y(ES) OR N(O)" @ INPUT NYSAT$
1190     IF NYSAT$="N" OR NYSAT$="NO" THEN GOTO 960
1200     IF NYSL$="N" OR NYSL$="NO" THEN GOTO 1400
1210     CLEAR @ DISP "COOLING REGION POINTS",JMIN,JTURN
1220     DISP "HEATING REGION POINTS",JTURN,JMAX
1230     ON KEY# 1,"COOLING" GOTO 1280
1240     ON KEY# 2,"HEATING" GOTO 1340
1250     DISP FNIVID$("SELECT OPTION") @ DISP @ DISP
1260     KEY LABEL
1270     GOTO 1270
1280     NSM=1 @ DISP "ENTER POINTS RANGE FOR COOLING SLOPE"
1290 DISP "ENTER 0,0 FOR HELP IN FINDING TURNING POINT IN HEATING CURVE" @ INPJ
 JMIN,JTURN
1300 IF JMIN=0 AND JTURN=0 THEN GOSUB Find
1310 DISP "JTURN=",JTURN
1320 DISP "ENTER JMIN,JTURN" @ INPUT JMIN,JTURN
1330     GOTO 1390
1340     NSM=2 @ DISP "ENTER POINTS RANGE FOR HEATING SLOPE"
1350 DISP "ENTER 0,0 FOR HELP IN FINDING TURNING POINT IN HEATING CURVE" @ INPJ
 JTURN,JMAX
1360 IF JTURN=0 AND JMAX=0 THEN GOSUB Find
1370 DISP "JTURN=",JTURN
1380 DISP "ENTER JTURN,JMAX" @ INPUT JTURN,JMAX
1390     NUMPLT=1 @ GOSUB Smooth
1400     GOSUB Aplot
1410     ON KEY# 1,"AGAIN" GOTO 1530
1420     ON KEY# 2,"DOSE" GOTO 1580
1430     ON KEY# 3,"PLOT" GOTO 1590
1440     ON KEY# 4,"SCRDMP" GOTO 3910
1450     ON KEY# 5,"EXIT" GOTO 3920
1460     CLEAR @ KEY LABEL
1470    DISP "SELECT OPTION - "&FNIVID$("DOSE")&" ALLOWS CALCULATION OF SAR"
1480    DISP "              - "&FNIVID$("AGAIN")&" RE-READS DATA FROM DISC"
1490    DISP "              - "&FNIVID$("PLOT")&" RE-PLOTS LAST PLOT - USE WITH C
ARE"
1500    DISP "              - "&FNIVID$("SCRDMP")&" DUMPS GRAPHICS TO PRINTER"
1510    DISP "              - "&FNIVID$("EXIT")&" TERMINATES EXECUTION"
1520     GOTO 1520
1530 CLEAR
1540 DISP "IF YOU CONTINUE DATA WILL BE RE-READ FROM DISC"
1550 DISP "PRESS "&FNIVID$("AGAIN")&"KEY IF YOU WISH TO DO THIS"
1560 ON KEY# 1,"AGAIN" GOTO 370 @ KEY LABEL
1570 GOTO 1570
1580 GOTO 930
1590     IF NUMSMO=0 THEN GOTO 1620
1600     GOSUB Aplot  @ GOTO 1520
1610 GOTO 1410
1620     DISP "OPTION NOT AVAILABLE" @ GOTO 370
1630     Smooth:
1640     ! ********************************
1650     ! *                              *
1660     ! *    CURVE SMOOTHING SECTION   *
1670     ! *                              *
1680     ! ********************************
1690     DISP "CURVE FITTING IN PROGRESS "&FNIVID$("PLEASE WAIT")
1700     NUMSMO=1
1710     IF NSM=2 THEN GOTO 1750
1720     N=JTURN-JMIN+1
1730     FOR I=1 TO N @ BTIME(I)=BTIME(JMIN+I-1) @ BTEMP(I)=BTEMP(JMIN+I-1) @ NEX
```

```
T I
1740     GOTO 1780
1750     N=JMAX-JTURN+1
1760     FOR I=1 TO JMAX-JTURN+1 @ BTIME(I)=BTIME(JTURN+I-1) @ BTEMP(I)=BTEMP(JTL
RN+I-1)
1770     NEXT I
1780     FOR I=1 TO 5
1790     R(I)=0 @ AA(I)=0 @ FOR J=1 TO 21 @ SN(I,J)=0 @ NEXT J @ NEXT I
1800     DISP "WHAT ORDER OF POLYNOMIAL DO YOU WISH TO FIT - 1=LINEAR..."
1810     INPUT NS
1820     FOR J=1 TO NS+1
1830     FOR I=1 TO N
1840     IF J=NS+1 AND BTIME(I)=0 THEN GOTO 1860
1850     R(J)=BTEMP(I)*BTIME(I)^(NS+1-J)+R(J) @ GOTO 1870
1860     R(J)=BTEMP(I)+R(J)
1870     NEXT I
1880     NEXT J
1890     FOR K=1 TO NS+1 @ FOR J=1 TO NS+1 @ FOR I=1 TO N
1900     IF 2*NS-J-K+2=0 AND BTIME(I)=0 THEN GOTO 1920
1910     SN(J,K)=BTIME(I)^(2*NS-J-K+2)+SN(J,K) @ GOTO 1930
1920     SN(J,K)=1+SN(J,K)
1930     NEXT I
1940     NEXT J
1950     NEXT K
1960     GOSUB Invt
1970     FOR I=1 TO NS+1 @ FOR J=1 TO NS+1
1980     AA(NS+2-I)=SN(NS+2-I,J)*R(J)+AA(NS+2-I) @ NEXT J @ NEXT I
1990     IF NSM=1 THEN DX=ATIME(JTURN)-ATIME(JMIN) ELSE DX=ATIME(JMAX)-ATIME(JTUR
N)
2000     IF NSM=1 THEN XSTART=ATIME(JMIN) ELSE XSTART=ATIME(JTURN)
2010     FOR K=1 TO 200 @ I=K+800 @ BTIME(I)=(K-1)*DX/200+XSTART @ BTEMP(I)=0 @ F
OR J=1 TO NS+1
2020     IF NS+1-J=0 AND BTIME(I)=0 THEN GOTO 2050
2030     BTEMP(I)=AA(J)*BTIME(I)^(NS+1-J)+BTEMP(I)
2040     GOTO 2060
2050     BTEMP(I)=AA(J)+BTEMP(I)
2060     NEXT J
2070     NEXT K
2080     RETURN
2090     Invt:
2100  !
2110  ! MATRIX INVERSION BY GAUSS-JORDAN ELIMINATION WITH FULL PIVOTING
2120  ! SQUARE MATRIX OF ORDER NP
2130  !
2140     DIM KP(5),JP(5)
2150     NP=NS+1
2160     NCOL=NP
2170  !
2180  ! INITIALIZE PERMUTATION VECTORS
2190  !
2200     FOR I=1 TO NP @ KP(I)=I @ JP(I)=I @ NEXT I
2210  !
2220  ! INVERT MATRIX
2230  !
2240     FOR IR=1 TO NP
2250  !
2260  ! FIND THE PIVOT ELEMENT
2270  !
2280     PIVOT=0
2290     FOR I=IR TO NP @ FOR J=IR TO NP @ K=KP(I) @ L=JP(J)
2300     TEST=ABS (SN(K,L))-ABS (PIVOT)
2310     IF TEST<=0 THEN GOTO 2370
```

```
2320     IF TEST>0 THEN GOTO 2340
2330     GOTO 2370
2340     IK=I
2350     JK=J
2360     PIVOT=SN(K,L)
2370     NEXT J @ NEXT I
2380   !
2390   ! THE PIVOT ELEMENT IS SN(K,L)
2400   !
2410   ! UPDATE PIVOT VECTORS
2420   !
2430     K=KP(IK) @ KP(IK)=KP(IR) @ KP(IR)=K @ L=JP(JK) @ JP(JK)=JP(IR) @ JP(IR)=
L
2440   !
2450   ! COMPUTE NEW ELEMENTS OF PIVOTAL ROW
2460   !
2470     FOR J=1 TO NCOL @ SN(K,J)=SN(K,J)/PIVOT @ NEXT J
2480   !
2490   ! COMPUTE REMAINING ELEMENTS OF IR'TH STEP
2500   !
2510     FOR I=1 TO NP
2520     IF I-K=0 THEN GOTO 2590
2530     AIL=SN(I,L)
2540     SN(I,L)=-(AIL/PIVOT)
2550     FOR J=1 TO NCOL
2560     IF J-L=0 THEN GOTO 2580
2570     SN(I,J)=SN(I,J)-AIL*SN(K,J)
2580     NEXT J
2590     NEXT I
2600   !
2610   !
2620   ! INVERT PIVOT ELEMENT
2630   !
2640     SN(K,L)=1/PIVOT @ NEXT IR
2650     RETURN
2660   Aplot:
2670     ! ************************
2680     ! *                      *
2690     ! *  PLOTTING SUBROUTINE *
2700     ! ************************
2710     !
2720 NLT=1
2730 CLEAR @ IF NUMPLT=0 THEN GOTO 2930
2740 ALPHA @ DISP "PARAMETERS OF FIT" @ DISP
2750 FOR I=1 TO NP @ DISP AA(I) @ NEXT I
2760 IF NSM=2 THEN GOTO 2790
2770 FOR I=1 TO NP @ COOL(I)=AA(I) @ NEXT I
2780 GOTO 2800
2790 FOR I=1 TO NP @ HEAT(I)=AA(I) @ NEXT I
2800 CHI2=0
2810 IF NTEST<2 THEN GOTO 2850
2820 SAR=4.2*(HEAT(1)-COOL(1))*1000
2830 IF NS=2 THEN SAR=2*SAR*ATIME(JTURN)+4.2*(HEAT(2)-COOL(2))
2840 DISP FNIVID$("SAR IS"),SAR,"W/kg"
2850 FOR I=1 TO N @ Y2=0 @ FOR J=1 TO NS+1
2860 IF NS+1-J=0 AND BTIME(I)=0 THEN GOTO 2880
2870 Y2=AA(J)*BTIME(I)^(NS+1-J)+Y2 @ GOTO 2890
2880 Y2=AA(J)+Y2
2890 NEXT J
2900 CHI2=(BTEMP(I)-Y2)^2/Y2+CHI2
2910 NEXT I
```

```
2920 DISP "OBSERVED CHI2= ",CHI2," WITH ",N+NS+1," D.F."
2930 DISP "DO YOU WANT A HARD COPY PLOT? Y(ES) OR N(O)"
2940 INPUT NY$@ IF NUMSMO=1 THEN GOTO 3040
2950 DISP "TIME WILL BE PLOTTED FROM ",XMIN,"TO",XMAX
2960 DISP "TEMPERATURE WILL BE PLOTTED FROM",YMIN,"TO",YMAX
2970 DISP "IF YOU ARE SATISFIED PRESS RETURN"
2980 INPUT NYLIM$
2990 IF NYLIM$="" THEN GOTO 3040
3000 DISP "ENTER TIME RANGE TO BE PLOTTED, TMIN,TMAX"
3010 INPUT XMIN,XMAX
3020 DISP "ENTER TEMPERATURE RANGE TO BE PLOTTED"
3030 INPUT YMIN,YMAX
3040 IF NTEST>= 1 THEN GOTO 3340
3050 CLEAR
3060 DISP "CALCULATING PLOT SCALING PARAMETERS "&FNIVID$("PLEASE WAIT")
3070 AXMAX=INT (LGT (XMAX))
3080 AYMAX=INT (LGT (YMAX))
3090 XF=10^AXMAX @ YF=10^AYMAX @ DELX=.01 @ DELY=.01
3100 XPWR$=VAL$ (AXMAX) @ YPWR$=VAL$ (AYMAX)
3110 ! XMIN=0 @ YMIN=0
3120 IF (XMAX-XMIN)/XF>= .1 THEN DELX=.02
3130 IF (XMAX-XMIN)/XF>= .3 THEN DELX=.05
3140 IF (XMAX-XMIN)/XF>= .5 THEN DELX=.1
3150 IF (XMAX-XMIN)/XF>= 1 THEN DELX=.2
3160 IF (XMAX-XMIN)/XF>= 3 THEN DELX=.5
3170 IF (XMAX-XMIN)/XF>= 5 THEN DELX=1
3180 IF (YMAX-YMIN)/YF>= .1 THEN DELY=.02
3190 IF (YMAX-YMIN)/YF>= .3 THEN DELY=.05
3200 IF (YMAX-YMIN)/YF>= .5 THEN DELY=.1
3210 IF (YMAX-YMIN)/YF>= 1 THEN DELY=.2
3220 IF (YMAX-YMIN)/YF>= 3 THEN DELY=.5
3230 IF (YMAX-YMIN)/YF>= 5 THEN DELY=1
3240 DISP "THE X-AXIS TICK SPACING WILL BE",DELX
3250 DISP "IF YOU ARE SATISFIED PRESS"&FNIVID$("ENDLINE")
3260 INPUT NTK$@ IF NTK$="" THEN GOTO 3280
3270 DISP "ENTER X-AXIS TICK SPACING" @ INPUT DELX
3280 DISP "THE Y-AXIS TICK SPACING WILL BE",DELY
3290 DISP "IF YOU ARE SATISFIED PRESS"&FNIVID$("ENDLINE")
3300 INPUT NTK$@ IF NTK$="" THEN GOTO 3320
3310 DISP "ENTER Y-AXIS TICK SPACING" @ INPUT DELY
3320 XNUM=CEIL ((XMAX-XMIN)/(XF*DELX))
3330 YNUM=CEIL ((YMAX-YMIN)/(YF*DELY))
3340 IF NY$="N" THEN GOTO 3380
3350 PLOTTER IS 505
3360 GRAPH @ IF NUMPLT=0 THEN GCLEAR @ DEG
3370 GOTO 3420
3380 PLOTTER IS 1
3390 GRAPH
3400 ! IF NUMPLT#0 AND NTEST>0 THEN GOTO 3110
3410 IF NUMPLT=0 THEN GCLEAR
3420 LOCATE 30,RATIO *100-10,20,95
3430 ! IF NUMPLT#0 AND NTEST>0 THEN GOTO 3110
3440 SCALE XMIN/XF,XMIN/XF+XNUM*DELX,YMIN/YF,YMIN/YF+YNUM*DELY
3450 IF NTEST>0 AND NUMPLT#0 THEN GOTO 3670
3460 FXD 2
3470 LGRID DELX,DELY,XMIN/XF,YMIN/YF
3480 AXS=(XMAX-XMIN)/(XF*10)
3490 AYS=(YMAX-YMIN)/(YF*10)
3500 MOVE (XMAX+XMIN)/(2*XF),YMIN/YF-2*AYS @ LORG 6
3510 LABEL USING "K" ; "TIME (SECS) x 1E"&XPWR$
3520 MOVE XMIN/XF-2*AXS,(YMAX+YMIN)/(2*YF)
```

```
3530 LORG 4 @ DEG @ LDIR 90
3540 LABEL USING "K" ; "TEMP.(DEGS C) x 1E"&YPWR$
3550 LDIR 0
3560 IF NPROBE=2 THEN GOTO 3630
3570 LINE TYPE 1
3580 MOVE ATIME(JMIN)/XF,ATEMP(JMIN)/YF
3590 FOR I=JMIN TO JMAX
3600 DRAW ATIME(I)/XF,ATEMP(I)/YF
3610 NEXT I
3620 IF NPROBE=1 THEN GOTO 3660
3630 LINE TYPE 5
3640 MOVE BTIME(KMIN)/XF,BTEMP(KMIN)/YF
3650 FOR I=KMIN TO KMAX @ DRAW BTIME(I)/XF,BTEMP(I)/YF @ NEXT I
3660 IF NYSL$="N" OR NYSL$="NO" THEN GOTO 3720
3670 LINE TYPE 4
3680 MOVE BTIME(801)/XF,BTEMP(801)/YF
3690 FOR I=801 TO 1000
3700 DRAW BTIME(I)/XF,BTEMP(I)/YF
3710 NEXT I
3720 PEN UP
3730 MOVE XMIN/XF,YMIN/YF
3740 IF SAR=0 THEN GOTO 3780
3750 MOVE XMIN/XF+4.5*AXS,YMAX/YF+.1*AYS
3760 LORG 4
3770 LABEL "SAR",SAR,"W/kg"
3780 NTEST=NTEST+1
3785 DISP "PRESS "&FNIVID$("CONTINUE")&"TO CALCULATE SAR"
3790 PAUSE
3800 KEY LABEL
3810 RETURN
3820 Find: ! *** SUBROUTINE TO FIND POINT WHERE HEATING STARTS
3830 DISP "ENTER A TEST RANGE J1,J2" @ INPUT J1,J2
3840 DISP "TIME","TEMP","DT/Dt"
3850 FOR I=J1 TO J2-1
3860 DISP ATIME(I),ATEMP(I),(ATEMP(I+1)-ATEMP(I))/(ATIME(I+1)-ATIME(I))
3870 NEXT I
3880 DISP "TRY ANOTHER RANGE - Y(ES) OR N(O)" @ INPUT NYR$
3890 IF NYR$="Y" OR NYR$="YES" THEN GOTO 3830
3900 DISP "ENTER JTURN" @ INPUT JTURN@ RETURN
3910 CHAIN "DUMP"
3920 END
```

PROGRAM FOR DATA ANALYSIS, CURVE SMOOTHING AND GRAPHICS - version B

```
! ************************************************
! *                                              *
! * PROGRAM TO READ TEMPERATURE READINGS TAKEN FROM *
! *  TWO NARDA PROBES AND STORED ON DISC. PLOT DATA *
! * IN VARIOUS FORMS AND DO DOSIMETRY CURVE FITTING *
! *                                              *
! * WRITTEN BY CHRISTOPHER C DAVIS. DECEMBER, 1986  *
! *                                              *
! ************************************************
OPTION BASE 1
MASS STORAGE IS ":D500"
DIM ATEMP(1000),ATIME(1000),BTEMP(1000),BTIME(1000),PONT(50),POFFT(50)
DIM ANUM(20),BNUM(20),IPON(20),IPOFF(20),DUMMY$[50]
! *** FUNCTION TO GIVE INVERSE VIDEO DISPLAYS
DEF FNIVID$(DUMMY$)
LENGTH=LEN (DUMMY$)
FOR II=1 TO LENGTH
DUMMY$[II,II]=CHR$ (NUM (DUMMY$[II,II])+128)
FNIVID$=DUMMY$
NEXT II
FN END
CLEAR
DISP FNIVID$("DATA DATE?")
DISP FNIVID$("DATE FORMAT")
DISP FNIVID$("DAY MONTH,YEAR")
INPUT EXPDATE$
DISP FNIVID$("ENTER SAMPLE ID")
INPUT SAMPLE$
EXPDATA$=EXPDATE$&SAMPLE$
ASSIGN# 1 TO  EXPDATA$&":D501"
NUMSMO=0 @ NTEST=0 @ SAR=0
IATOT=0 @ IBTOT=0 @ ION=0 @ IOFFT=0 @ IA1=1 @ IB1=1 @ ION1=1 @ IOFF1=1
READ# 1 ; IATOT ! READ NO OF PROBE A READINGS
FOR I=IA1 TO IATOT @ READ# 1 ; ATEMP(I),ATIME(I)@ NEXT I ! PROBE A DATA
READ# 1 ; IBTOT ! READ NO OF PROBE B READINGS
FOR I=IB1 TO IBTOT @ READ# 1 ; BTEMP(I),BTIME(I)@ NEXT I ! PROBE B DATA
READ# 1 ; ION ! NO OF TIMES MW PWR TURNED ON
FOR I=ION1 TO ION @ READ# 1 ; PONT(I)@ NEXT I ! ON TIMES
READ# 1 ; IOFFT ! NO OF TIMES MW PWR TURNED OFF
FOR I=IOFF1 TO IOFFT @ READ# 1 ; POFFT(I)@ NEXT I ! OFF TIMES
IA1=IA1+IATOT @ IB1=IB1+IBTOT @ ION1=ION1+ION @ IOFF1=IOFF1+IOFFT
ASSIGN# 1 TO  *
! PURGE EXPDATA$&":D501"
 DISP "TOTAL PROBE A READINGS = ",IATOT
! FOR I=1 TO IATOT @ DISP ATEMP(I),ATIME(I),I @ NEXT I
 DISP "TOTAL PROBE B READINGS = ",IBTOT
! FOR I=1 TO IBTOT @ DISP BTEMP(I),BTIME(I),I @ NEXT I
DISP "MW POWER TURNED ON ",ION," TIMES"
DISP "MW POWER TURNED OFF ",IOFFT," TIMES"
DISP "THE MW PWR WAS TURNED ON AT THE FOLLOWING TIMES"
FOR I=1 TO ION @ DISP PONT(I) @ NEXT I
DISP "Sample ID is ",FNIVID$(SAMPLE$)
DISP "Date of exposure  was ",FNIVID$(EXPDATE$)
  ! ************************************************
  ! *                                              *
  ! *            SECTION FOR SMOOTHING             *
  ! *      AND PLOTTING OF EXPERIMENTAL DATA       *
  ! *                                              *
  ! *  WRITTEN BY Christopher C. Davis, June 1984. *
  ! *          MODIFIED DECEMBER 1986              *
  ! *                                              *
```

```
610    ! ************************************************
620    !
630    !
640     DIM AA(5),SN(21,21),R(20),HEAT(5),COOL(5) ! ARRAYS USED IN CURVE SMOOTHI
G
650     NYSL$="N"
660     IF ATIME(IATOT)>BTIME(IBTOT) THEN XMAX=ATIME(IATOT) ELSE XMAX=BTIME(IBTO
) ! SET X AXIS MAX TO MAX OBSERVATION TIME
670     XMIN=0 ! INITIALLY SET X ORIGIN TO 0
680     JMIN=1 @ KMIN=1
690     DISP "TEMPERATURE MEASUREMENT INTERVAL WAS",XMAX
700     YMIN=10^20 @ YMAX=-(10^20)
710 ! *** FIND MAXIMA AND MINIMA OF DATA
720     FOR I=1 TO IATOT
730     IF ATEMP(I)>YMAX THEN YMAX=ATEMP(I)
740     IF ATEMP(I)<YMIN THEN YMIN=ATEMP(I)
750     NEXT I
760     FOR I=1 TO IBTOT
770     IF BTEMP(I)>YMAX THEN YMAX=BTEMP(I)
780     IF BTEMP(I)<YMIN THEN YMIN=BTEMP(I)
790     NEXT I
800     NUMPLT=0 @ IF NTEST#0 THEN CLEAR
810     DISP "DO YOU WISH TO PLOT PROBE A, PROBE B, OR BOTH? "&FNIVID$("ENTER 1,
 OR 3")
820     DISP "YOU CAN ONLY CALCULATE SAR USING PROBE A"
830     INPUT NPROBE
840     IF NPROBE=1 THEN GOTO 860
850     GOTO 870
860     FOR I=1 TO IATOT @ BTIME(I)=ATIME(I) @ BTEMP(I)=ATEMP(I) @ NEXT I
870     IF NTEST=0 THEN GOTO 890
880     NYSL$="Y"
890     DISP "ENTER TIME REGION YOU WISH TO ANALYZE"
900     INPUT TMIN,TMAX
910     FOR I=1 TO IATOT ! FIND RANGE OF TEMP ARRAY TO BE PLOTTED
920     IF TMIN>ATIME(I) THEN JMIN=I
930     NEXT I
940     FOR I=1 TO IBTOT @ IF TMIN>BTIME(I) THEN KMIN=I
950     NEXT I
960     IF ATIME(JMIN)<BTIME(KMIN) THEN TMIN=ATIME(JMIN) ELSE TMIN=BTIME(KMIN)
970     FOR I=1 TO IATOT @ IF TMAX>ATIME(I) THEN JMAX=I
980     NEXT I
990     FOR I=1 TO IBTOT @ IF TMAX>BTIME(I) THEN KMAX=I
1000     NEXT I
1010     IF ATIME(JMAX)>BTIME(KMAX) THEN TMAX=ATIME(JMAX) ELSE TMAX=BTIME(KMAX)
1020     IF NTEST=0 THEN GOTO 1250
1030     IF NYSL$="N" OR NYSL$="NO" THEN GOTO 1250
1040 ! *** SECTION FOR SELECTION OF CURVE REGION FOR SMOOTHING
1050     TEMAX=-1.E20 @ TEMIN=1.E20
1060     FOR I=JMIN TO JMAX @ IF ATEMP(I)<TEMIN THEN TEMIN=ATEMP(I)
1070     IF ATEMP(I)=TEMIN THEN JTURN=I ! Find inflection point on temp curve
1080     NEXT I
1090     DISP "NUMBER OF POINTS ON COOLING SLOPE= ",JTURN-JMIN+1
1100     DISP "NUMBER OF POINTS ON HEATING SLOPE= ",JMAX-JTURN+1
1110     DISP "IS THIS SATISFACTORY? Y(ES) OR N(O)" @ INPUT NYSAT$
1120     IF NYSAT$="N" OR NYSAT$="NO" THEN GOTO 890
1130     IF NYSL$="N" OR NYSL$="NO" THEN GOTO 1250
1140     CLEAR @ DISP "COOLING REGION POINTS",JMIN,JTURN
1150     DISP "HEATING REGION POINTS",JTURN,JMAX
1160     ON KEY# 1,"COOLING" GOTO 1210
1170     ON KEY# 2,"HEATING" GOTO 1230
1180     DISP FNIVID$("SELECT OPTION") @ DISP @ DISP
1190     ! ALL
```

```
1200    GOTO 1200
1210    NSM=1 @ DISP "ENTER POINTS RANGE FOR COOLING SLOPE" @ DISP @ DISP @ INPU
T JMIN,JTURN
1220    GOTO 1240
1230    NSM=2 @ DISP "ENTER POINTS RANGE FOR HEATING SLOPE" @ DISP @ DISP @ INPU
T JTURN,JMAX
1240    NUMPLT=1 @ GOSUB Smooth
1250    GOSUB Aplot
1260    ON KEY# 1,"AGAIN" GOTO 1380
1270    ON KEY# 2,"DOSE" GOTO 1430
1280    ON KEY# 3,"PLOT" GOTO 1440
1290    ON KEY# 4,"SCRDMP" GOTO 3530
1300    ON KEY# 5,"EXIT" GOTO 3540
1310    CLEAR @ KEY LABEL
1320   DISP "SELECT OPTION - "&FNIVID$("DOSE")&" ALLOWS CALCULATION OF SAR"
1330   DISP "               - "&FNIVID$("AGAIN")&" RE-READS DATA FROM DISC"
1340   DISP "               - "&FNIVID$("PLOT")&" RE-PLOTS LAST PLOT - USE WITH C
ARE"
1350   DISP "               - "&FNIVID$("SCRDMP")&" DUMPS GRAPHICS TO PRINTER"
1360   DISP "               - "&FNIVID$("EXIT")&" TERMINATES EXECUTION"
1370    GOTO 1370
1380 CLEAR
1390 DISP "IF YOU CONTINUE DATA WILL BE RE-READ FROM DISC"
1400 DISP "PRESS "&FNIVID$("AGAIN")&"KEY IF YOU WISH TO DO THIS"
1410 ON KEY# 1,"AGAIN" GOTO 300 @ KEY LABEL
1420 GOTO 1420
1430 GOTO 860
1440    IF NUMSMO=0 THEN GOTO 1470
1450    GOSUB Aplot  @ GOTO 1370
1460 GOTO 1260
1470    DISP "OPTION NOT AVAILABLE" @ GOTO 300
1480    Smooth:
1490    ! ********************************
1500    ! *                              *
1510    ! *    CURVE SMOOTHING SECTION   *
1520    ! *                              *
1530    ! ********************************
1540    DISP "CURVE FITTING IN PROGRESS "&FNIVID$("PLEASE WAIT")
1550    NUMSMO=1
1560    IF NSM=2 THEN GOTO 1600
1570    N=JTURN-JMIN+1
1580    FOR I=1 TO N @ BTIME(I)=BTIME(JMIN+I-1) @ BTEMP(I)=BTEMP(JMIN+I-1) @ NEX
T I
1590    GOTO 1630
1600    N=JMAX-JTURN+1
1610    FOR I=1 TO JMAX-JTURN+1 @ BTIME(I)=BTIME(JTURN+I-1) @ BTEMP(I)=BTEMP(JTU
RN+I-1)
1620    NEXT I
1630    FOR I=1 TO 5
1640    R(I)=0 @ AA(I)=0 @ FOR J=1 TO 21 @ SN(I,J)=0 @ NEXT J @ NEXT I
1650    DISP "WHAT ORDER OF POLYNOMIAL DO YOU WISH TO FIT - 1=LINEAR..."
1660    INPUT NS
1670    FOR J=1 TO NS+1
1680    FOR I=1 TO N
1690    IF J=NS+1 AND BTIME(I)=0 THEN GOTO 1710
1700    R(J)=BTEMP(I)*BTIME(I)^(NS+1-J)+R(J) @ GOTO 1720
1710    R(J)=BTEMP(I)+R(J)
1720    NEXT I
1730    NEXT J
1740    FOR K=1 TO NS+1 @ FOR J=1 TO NS+1 @ FOR I=1 TO N
1750    IF 2*NS-J-K+2=0 AND BTIME(I)=0 THEN GOTO 1770
1760    SN(J,K)=SN(J,K)+BTIME(I)^(2*NS-J-K+2)+SN(J,K)
```

```
1770     SN(J,K)=1+SN(J,K)
1780     NEXT I
1790     NEXT J
1800     NEXT K
1810     GOSUB Invt
1820     FOR I=1 TO NS+1 @ FOR J=1 TO NS+1
1830     AA(NS+2-I)=SN(NS+2-I,J)*R(J)+AA(NS+2-I) @ NEXT J @ NEXT I
1840     IF NSM=1 THEN DX=ATIME(JTURN)-ATIME(JMIN) ELSE DX=ATIME(JMAX)-ATIME(JT
N)
1850     IF NSM=1 THEN XSTART=ATIME(JMIN) ELSE XSTART=ATIME(JTURN)
1860     FOR K=1 TO 200 @ I=K+800 @ BTIME(I)=(K-1)*DX/200+XSTART @ BTEMP(I)=0 @
OR J=1 TO NS+1
1870     IF NS+1-J=0 AND BTIME(I)=0 THEN GOTO 1900
1880     BTEMP(I)=AA(J)*BTIME(I)^(NS+1-J)+BTEMP(I)
1890     GOTO 1910
1900     BTEMP(I)=AA(J)+BTEMP(I)
1910     NEXT J
1920     NEXT K
1930     RETURN
1940     Invt:
1950  !
1960  ! MATRIX INVERSION BY GAUSS-JORDAN ELIMINATION WITH FULL PIVOTING
1970  ! SQUARE MATRIX OF ORDER NP
1980  !
1990     DIM KP(5),JP(5)
2000     NP=NS+1
2010     NCOL=NP
2020  !
2030  ! INITIALIZE PERMUTATION VECTORS
2040  !
2050     FOR I=1 TO NP @ KP(I)=I @ JP(I)=I @ NEXT I
2060  !
2070  ! INVERT MATRIX
2080  !
2090     FOR IR=1 TO NP
2100  !
2110  ! FIND THE PIVOT ELEMENT
2120  !
2130     PIVOT=0
2140     FOR I=IR TO NP @ FOR J=IR TO NP @ K=KP(I) @ L=JP(J)
2150     TEST=ABS(SN(K,L))-ABS(PIVOT)
2160     IF TEST<= 0 THEN GOTO 2220
2170     IF TEST>0 THEN GOTO 2190
2180     GOTO 2220
2190     IK=I
2200     JK=J
2210     PIVOT=SN(K,L)
2220     NEXT J @ NEXT I
2230  !
2240  ! THE PIVOT ELEMENT IS SN(K,L)
2250  !
2260  ! UPDATE PIVOT VECTORS
2270  !
2280     K=KP(IK) @ KP(IK)=KP(IR) @ KP(IR)=K @ L=JP(JK) @ JP(JK)=JP(IR) @ JP(IR)
L
2290  !
2300  ! COMPUTE NEW ELEMENTS OF PIVOTAL ROW
2310  !
2320     FOR J=1 TO NCOL @ SN(K,J)=SN(K,J)/PIVOT @ NEXT J
2330  !
2340  ! COMPUTE REMAINING ELEMENTS OF IR'TH STEP
```

```
2350  !
2360    FOR I=1 TO NP
2370    IF I-K=0 THEN GOTO 2440
2380    AIL=SN(I,L)
2390    SN(I,L)=-(AIL/PIVOT)
2400    FOR J=1 TO NCOL
2410    IF J-L=0 THEN GOTO 2430
2420    SN(I,J)=SN(I,J)-AIL*SN(K,J)
2430    NEXT J
2440    NEXT I
2450  !
2460  !
2470  ! INVERT PIVOT ELEMENT
2480  !
2490    SN(K,L)=1/PIVOT @ NEXT IR
2500    RETURN
2510  Aplot:
2520    ! ************************
2530    ! *                      *
2540    ! *  PLOTTING SUBROUTINE *
2550    ! ************************
2560    !
2570 NLT=1
2580 CLEAR @ IF NUMPLT=0 THEN GOTO 2780
2590 ALPHA @ DISP "PARAMETERS OF FIT" @ DISP
2600 FOR I=1 TO NP @ DISP AA(I) @ NEXT I
2610 IF NSM=2 THEN GOTO 2640
2620 FOR I=1 TO NP @ COOL(I)=AA(I) @ NEXT I
2630 GOTO 2650
2640 FOR I=1 TO NP @ HEAT(I)=AA(I) @ NEXT I
2650 CHI2=0
2660 IF NTEST<2 THEN GOTO 2700
2670 SAR=4.2*(HEAT(1)-COOL(1))
2680 IF NS=2 THEN SAR=2*SAR*ATIME(JTURN)+4.2*(HEAT(2)-COOL(2))
2690 DISP FNIVID$("SAR IS"),SAR,"Watts per gram"
2700 FOR I=1 TO N @ Y2=0 @ FOR J=1 TO NS+1
2710 IF NS+1-J=0 AND BTIME(I)=0 THEN GOTO 2730
2720 Y2=AA(J)*BTIME(I)^(NS+1-J)+Y2 @ GOTO 2740
2730 Y2=AA(J)+Y2
2740 NEXT J
2750 CHI2=(BTEMP(I)-Y2)^2/Y2+CHI2
2760 NEXT I
2770 DISP "OBSERVED CHI2= ",CHI2," WITH ",N+NS+1," D.F."
2780 DISP "DO YOU WANT A HARD COPY PLOT? Y(ES) OR N(O)"
2790 INPUT NY$@ IF NUMSMO=1 THEN GOTO 2890
2800 DISP "TIME WILL BE PLOTTED FROM ",XMIN,"TO",XMAX
2810 DISP "TEMPERATURE WILL BE PLOTTED FROM",YMIN,"TO",YMAX
2820 DISP "IF YOU ARE SATISFIED PRESS RETURN"
2830 INPUT NYLIM$
2840 IF NYLIM$="" THEN GOTO 2890
2850 DISP "ENTER TIME RANGE TO BE PLOTTED, TMIN,TMAX"
2860 INPUT XMIN,XMAX
2870 DISP "ENTER TEMPERATURE RANGE TO BE PLOTTED"
2880 INPUT YMIN,YMAX
2890 IF NTEST>= 1 THEN GOTO 3050
2900 CLEAR
2910 DISP "CALCULATING PLOT SCALING PARAMETERS "&FNIVID$("PLEASE WAIT")
2920 AXMAX=INT (LGT (XMAX))
2930 AYMAX=INT (LGT (YMAX))
2940 XF=10^AXMAX @ YF=10^AYMAX
2950 XPWR$=VAL$ (AXMAX) @ YPWR$=VAL$ (AYMAX)
2960 ! XM[illegible]
```

```
2970 IF XMAX/XF>= 1 THEN DELX=.2
2980 IF XMAX/XF>= 3 THEN DELX=.5
2990 IF XMAX/XF>= 5 THEN DELX=1
3000 IF YMAX/YF>= 1 THEN DELY=.2
3010 IF YMAX/YF>= 3 THEN DELY=.5
3020 IF YMAX/YF>= 5 THEN DELY=1
3030 XNUM=CEIL ((XMAX-XMIN)/(XF*DELX))
3040 YNUM=CEIL ((YMAX-YMIN)/(YF*DELY))
3050 IF NYS="N" THEN GOTO 3090
3060 PLOTTER IS 505
3070 GRAPH @ IF NUMPLT=0 THEN GCLEAR @ DEG
3080 GOTO 3130
3090 PLOTTER IS 1
3100 GRAPH
3110 ! IF NUMPLT#0 AND NTEST>0 THEN GOTO 3110
3120 IF NUMPLT=0 THEN GCLEAR
3130 LOCATE 30,RATIO *100-10,20,95
3140 ! IF NUMPLT#0 AND NTEST>0 THEN GOTO 3110
3150 SCALE XMIN/XF,XMIN/XF+XNUM*DELX,YMIN/YF,YMIN/YF+YNUM*DELY
3160 IF NTEST>0 AND NUMPLT#0 THEN GOTO 3380
3170 FXD 1
3180 LGRID DELX,DELY,XMIN/XF,YMIN/YF
3190 AXS=(XMAX-XMIN)/(XF*10)
3200 AYS=(YMAX-YMIN)/(YF*10)
3210 MOVE XMIN/XF+4.5*AXS,YMIN/YF-2*AYS @ LORG 6
3220 LABEL USING "K" ; "TIME (SECS) x 1E"&XPWR$
3230 MOVE XMIN/XF-2*AXS,YMIN/YF+4.5*AYS
3240 LORG 4 @ DEG @ LDIR 90
3250 LABEL USING "K" ; "TEMP.(DEGS C) x 1E"&YPWR$
3260 LDIR 0
3270 IF NPROBE=2 THEN GOTO 3340
3280 LINE TYPE 1
3290 MOVE ATIME(JMIN)/XF,ATEMP(JMIN)/YF
3300 FOR I=JMIN TO JMAX
3310 DRAW ATIME(I)/XF,ATEMP(I)/YF
3320 NEXT I
3330 IF NPROBE=1 THEN GOTO 3370
3340 LINE TYPE 5
3350 MOVE BTIME(KMIN)/XF,BTEMP(KMIN)/YF
3360 FOR I=KMIN TO KMAX @ DRAW BTIME(I)/XF,BTEMP(I)/YF @ NEXT I
3370 IF NYSL$="N" OR NYSL$="NO" THEN GOTO 3430
3380 LINE TYPE 4
3390 MOVE BTIME(801)/XF,BTEMP(801)/YF
3400 FOR I=801 TO 1000
3410 DRAW BTIME(I)/XF,BTEMP(I)/YF
3420 NEXT I
3430 PEN UP
3440 MOVE XMIN/XF,YMIN/YF
3450 IF SAR=0 THEN GOTO 3490
3460 MOVE XMIN/XF+4.5*AXS,YMAX/YF+.1*AYS
3470 LORG 4
3480 LABEL "SAR",SAR,"Watts per gram"
3490 NTEST=NTEST+1
3500 PAUSE
3510 KEY LABEL
3520 RETURN
3530 CHAIN "DUMP"
3540 END
```

Attachment #2

IRPA 7

Seventh International Congress of the
International Radiation Protection Association

Sydney, 10-17 April 1988

RADIATION PROTECTION PRACTICE

VOLUME I

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AN AUTOMATED DOSIMETRY SYSTEM FOR MICROWAVE AND THERMAL EXPOSURE OF BIOLOGICAL SAMPLES *IN VITRO*

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INTRODUCTION

Dielectric heating is a recognized mechanism for the induction of radiofrequency (RF) bioeffects considered to be thermal in origin - that is attributable to temperature increases. However, apart from RF-heat cell killing (e.g. Sapareto et al.,1982, Chang et al.,1987) very little information is available on quantitative relationships between RF bioeffects at the cellular and molecular level and temperature profiles over time or thermal dosage (TD). The determination of RF heating/cooling curves can be used to compute the specific absorption rate (SAR) and the specific absorption (SA) in an exposed sample (Stuchly and Stuchly, 1986). Biological variables and exposure conditions can be controlled in experiments with *in vitro* systems to a degree not achievable *in vivo*. An attractive model to study RF bioeffects is the transformation of lymphocytes *in vitro*, provided the biological variables can be related to dosimetric quantities that characterize exposure (Czerski,1975; Budd and Czerski,1985). To accomplish this, we designed an exposure system with provision for real-time temperature monitoring with RF-field non-perturbing temperature probes. The exposure system, which will be described in detail later, has multiple sample chambers. To allow on-line thermometry and dosimetry, one of these chambers is used as a site for a non-perturbing temperature probe. From the temperature (T)/time(t) history of the sample chamber, an exposure dosage can be determined and described in terms of TD, SAR, SA, or electric field strength. The system can be used for studies of RF-bioeffects in any tissue culture cell line or other *in vitro* biological sample. The monitoring component can be applied to the study of temperature-dependent effects, irrespective of the modality used for heating.

EXPOSURE SYSTEM

A schematic diagram of the system for microwave exposure of samples is shown in Fig. 1.

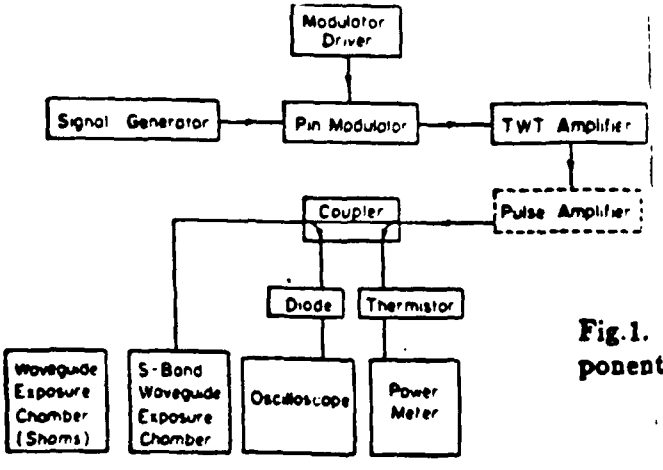

Fig.1. Schematic diagram of essential components of microwave exposure system.

The output from a CW Hewlett-Packard Model 8616A oscillator feeds a Hughes Model 1177H TWT amplifier. Pulsed or amplitude-modulated waveforms are obtained through the use of a Hewlett-Packard Model 8403A p-i-n modulator and driver. For high peak power, but low duty-cycle, pulsed exposures the signal can be further amplified with a 1kW amplifier (MCL, La Grange, Ill.) The amplified signal enters a shorted section of S-band rectangular waveguide 300 mm long through a matched coaxial feed. The waveguide sample holder receives its power through an isolator (not shown specifically in Fig. 1) and a dual-directional coupler that allows forward and reflected powers to be measured with Hewlett-Packard Model 432A power meters, and waveforms to be monitored. A sample holder for biological specimens is supported centrally in the waveguide in a block of low-density polystyrene that fills the cross-section of the guide. An identical waveguide section with an identical plastic sample holder is used for sham exposures. During microwave exposures, both waveguide assemblies were housed in a CO_2 tissue culture incubator thermostated at 37.0°C. Various sample holders were tested: a 4-chamber plastic tissue culture slide (Miles Scientific # 4804) was found satisfactory in our application. Each chamber holds 1 ml. A miniature thermistor temperature probe enters each waveguide section through a small hole in the top shorting plate. The hole is drilled near the guide wall, at the center of the shorter dimension – this is a low electric field point and the hole produces minimal disturbance. The active end of the thermistor probe enters the sample in one of the 4 chambers of the tissue culture dish. We could move the probe from chamber to chamber to check exposure uniformity. Two types of non-field perturbing probe have been used in this way: A Narda Model 8011B non-perturbing double temperature probe or two Vitek Model 101 probes. The temperatures in both the exposed and sham-exposed samples are recorded continuously during an experimental run. The temperature probes are connected to a Hewlett-Packard relay activator, Model 59306A, which is itself connected to a Keithley Model 192 DVM. Both the relay activator and DVM are under the control of a Hewlett-Packard Model 86 desk-top computer through the HPIB (IEEE-488 bus)(Fig.2).

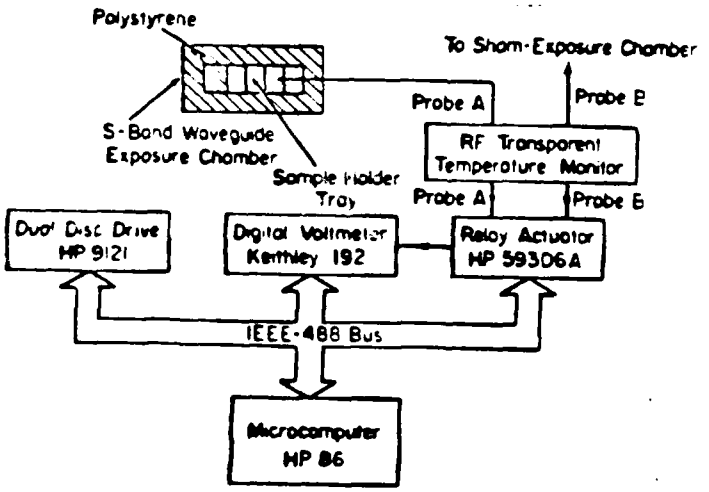

Fig.2. Schematic diagram of multiple-chamber sample holder with non-perturbing temperature measurement and automated dosimetry system.

Under computer control, the temperatures of exposed and sham-exposed samples are recorded sequentially and stored in memory. Since the temperature of sham-exposed samples is essentially constant, it is monitored less frequently than the temperature of the exposed samples. A typical experimental protocol would involve 10 temperature readings of the exposed sample for every one reading of the sham-exposed. For conventional thermal exposures, the temperature can be elevated by increasing the temperature of the incubator.

TEMPERATURE/TIME ANALYSIS

During a typical experiment, the temperature is recorded at regular intervals, with a minimum measurement interval $< 1s$, before the beginning of, during, and after the exposure. The "on" and "off" times of exposure are recorded on the computer by the operator using a "soft-key" interrupt capability. At the conclusion of a run, the T(t) behavior is analyzed to determine SAR.

If the SAR is S (Wkg^{-1}) and the specific heat of the sample is $C(Jkg^{-1}K^{-1})$, then the SAR can be determined from the change in heating rate when microwave power is applied.

$$\left(\frac{dT}{dt}\right)_S - \left(\frac{dT}{dt}\right)_{S=0} = \frac{S}{C}$$

In practice, this analysis is performed automatically. At the end of a run, the point of inflection, or turning point, in T(t) is found numerically. The temperature profile to the left and right of this point is fitted by least-squares to a linear or quadratic function and the change in slope at the beginning of the exposure period yields the SAR. Figures 3 and 4 are examples of such a procedure.

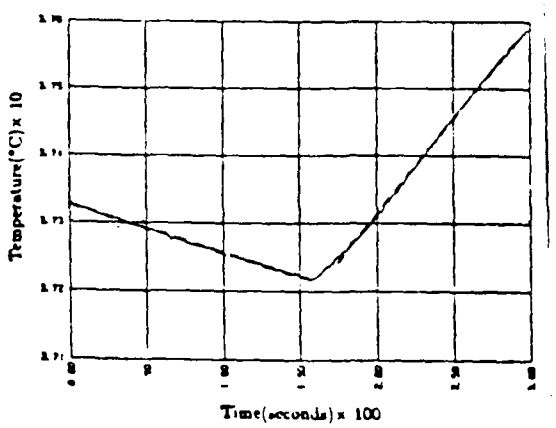

Fig.3. Temperature/time history of an exposed sample showing linear fits to cooling and heating portions of curve for dosimetry.

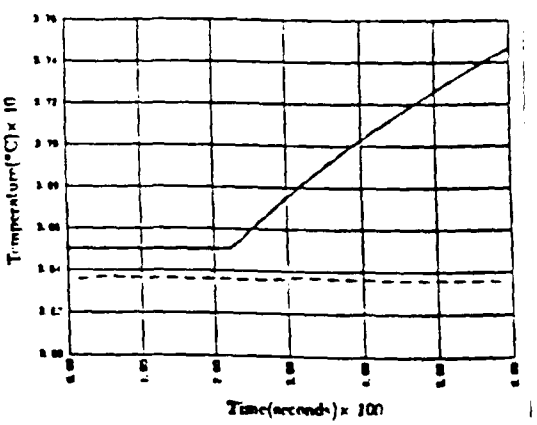

Fig.4. Temperature/time history of exposed (upper curve) and sham-exposed samples (lower curve). The dosimetry for the exposed sample has been determined from a quadratic fit to the heating curve during microwave exposure and a linear fit to the equilibrated portion of the curve prior to exposure.

Figure 3 shows the temperature/time history of a sample that was cooling prior to the start of microwave exposure. The linear fits to the cooling and heating portions of the curve yield the SAR. Figure 4. shows the heating of a sampled that was quite well equilibrated before exposure. Because the signal/noise ratio is high the SAR was determined by a quadratic fit to the heating portion of the curve. The quality of the fit near the turning point can always be examined to determine whether the slope value is realistic. However,unless the temperature/time profile has very low noise, it is generally better to use the linear fit to T(t) near the turning point or a biased estimate of the slope can result. Figure 4 also shows the temperature/time history of the sham-exposed sample to illustrate its temperature stability.

RESULTS AND DISCUSSION

The advantages of the procedure described above are severalfold. The sample need not be equilibrated before SAR is determined. Repeated determination of SAR can easily and quickly be made, which allows the SAR uniformity from one sample chamber to another to be determined. Exact knowledge of the microwave power is not required: reproducible exposures can be made at known SAR by using the measured forward power corresponding to a given SAR measurement, provided the experimental arrangement is not altered between exposures. Dosimetry is not affected by other losses in the system. The waveguide exposure system with four sample chambers was found to give an SAR uniformity from one chamber to the other within 10%. Thus, experimental samples can be exposed and examined in triplicate, the fourth chamber being used for the insertion of the temperature probe.

The system is biocompatible and, depending on cell line, cell density, and medium, allows continuous exposures of several days duration. The biocompatibility of this arrangement was proven by studying the growth of human lymphocytes under various conditions within the waveguide. As well as providing biocompatible exposure conditions, the system is flexible: it allows the exposure of tissue culture cells growing in suspension or in monolayers, and can easily be used with cell-free samples. The exposure system is relatively simple, and can be assembled from off-the-shelf components. Its capabilities can be expanded by introducing computer control of RF power input based on feedback from temperature measurements.

ACKNOWLEDGEMENTS

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RADIATION PROTECTION PRACTICE

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EFFECTS OF 2450 MHz EXPOSURE ON HUMAN LYMPHOCYTE TRANSFORMATION IN VITRO

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and

E. Manikowska-Czerska and C.C. Davis
Electrical Engineering Department
University of Maryland

and

P. Czerski
CDRH/FDA, Rockville, MD, USA

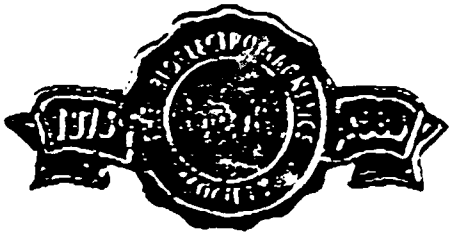
ABSTRACT

There are contradictory reports in the literature on the effects of microwave exposure on transformation of lymphocytes. To resolve this question, human lymphocytes have been exposed in vitro to 2450 MHz radiation under carefully controlled conditions at various power levels and for periods up to 120 hours. Lymphocyte tranformation in exposed samples was compared to control samples, sham-exposed or incubated in a conventional tissue culture incubator. Heparinized peripheral blood samples were obtained by venipuncture from healthy volunteers, lymphocytes were separated by gradient centrifugation (Lymphocyte Separation Medium, Litton Bionetics) and incubated in chromosome medium 1A without phytohemagglutinin (Gibco) at a standard concentration of 10^6 cells/ml medium. Using the automated dosimetry microwave exposure system described in a companion paper (Joyner et al., this Congress) the temperature profile over time was recorded for each sample, and average and peak specific absorption rates (SAR), specific absorption (SA), and thermal dosage (TD) were calculated. At harvest, cells were counted and their viability was tested using neutral red, Janus green, and trypan blue staining. Standardized cytological preparation were made using a "Cytospin II" (Shandon) cytocentrifuge, air-dried, fixed in methanol and stained with a combination of Wright and Giemsa stains. The percentages of untransformed (small) lymphocytes, intermediate and lymphoblastoid cells were determined, based on morphological features, quantitated using an "Optomax" image analyzer. The correlation of lymphocyte transformation with SAR, SA, and TD was examined. The implications of the results for a "thermal" vs "nonthermal" mechanism of interaction will be discussed.

Attachment #3

THE BIOELECTROMAGNETICS SOCIETY

TENTH ANNUAL MEETING 1988



ABSTRACTS

Presented at:

Westin Hotel
Stamford, Connecticut
June 19-23, 1988

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B-08-5 MICROWAVE-INDUCED DESENSITIZATION OF ACETYLCHOLINE RECEPTOR CHANNEL IN CULTURED QUAIL MYOTUBES. P. Bernardi*, G. D'Inzeo*, F. Eusebi*, F. Grassi*, C. Tamburello*. Department of Electronics, University of Rome "La Sapienza", 00184 Rome.

The effects of microwaves on the acetylcholine (ACh) receptor channel have been examined recording ACh-induced currents. Experiments were performed on single 3-5 day old unstriated and non-innervated quail myotubes. The properties of single ACh activated channels were investigated using the patch-clamp technique with high resistance seals between the membrane and the pipette in cell attached configuration. Sylgard coated pipettes were filled with acetylcholine at the concentration 5×10^{-8} M. Single channel were analyzed by the threshold crossing method. CW microwaves (7 mW at 10.75 GHz) were applied using a horn antenna (16 dB gain) at a distance of 25 cm. from the sample, 30° inclinated to the ground and with the E vector lying on the plane of incidence. In the control, before the exposure, ACh-activated channel current of 1.74 $\pm$ 0.01 pA (mean $\pm$ s.e.m.; 740 events), a mean channel open time of 3.46 $\pm$ 0.13 ms, a mean closed time of 18.7 $\pm$ 1.3 ms were measured. When myotubes were exposed to MW for 120 s, the closed time increased to 50.1 $\pm$ 5.1 ms (300) within 90s from the onset. Channel open time (3.43 $\pm$ 0.16 ms) and current (1.77 $\pm$ 0.01 pA) were not affected by irradiation. After the end of exposure a partial recovery of the activity was obtained. It is concluded that exposure to microwaves increases the rate of desensitization and decreases the channel opening probability. Other experiments indicate that this effect develops only within range of field intensity.

B-08-6 EFFECTS OF 2.45 GHz EXPOSURE ON SPONTANEOUS HUMAN LYMPHOCYTE TRANSFORMATION IN VITRO. E C Elson, Walter Reed AIR, Silver Spring, MD 20905. E M Czerska,* C C. Davis, Electrical Engineering Dept., University of Maryland., College Park, MD 20742, P. Czerski, CDRH/FDA, Rockville, MD 20857.

Human peripheral blood lymphocytes in chromosome medium 1A without PHA (Gibco) have been exposed to continuous (CW) or pulsed (PW) 2.45 GHz microwaves using the automated dosimetry exposure system described in a companion paper (Joyner et al., this meeting) at heating and non-heating SAR levels for periods up to 120 hours. At harvest, standardized cytological preparations were made using a cytospin II cytocentrifuge, air-dried, fixed in methanol and stained with Wright/Giemsa stain. The percentages of untransformed lymphocytes, intermediate and transformed lymphoblastoid cells were determined based on morphological features quantitated using an "Optomax" image analyser and compared between exposed (CW or PW) and sham-exposed samples. CW or PW exposure at the same average SAR resulting in similar temperature profiles over time affects lymphocyte transformation differently. Mechanistic implications of this finding are discussed.

PW-05 AUTOMATED DOSIMETRY OF MICROWAVE AND THERMAL EXPOSURE OF BIOLOGICAL SAMPLES IN VITRO. K.H. Joyner,* Telecom, Melbourne, Australia. E.C. Elson, Walter Reed AIR, Silver Spring, MD 20905. C.C. Davis, E.M. Czerska,* Electrical Eng. Dept., U. of MD., College Park, MD 20742, P. Czerski, CDRH/FDA, Rockville, MD 20857.

A microwave exposure and dosimetric system will be described that provides non-perturbing automated recording of the temperature history of exposed and control samples in vitro. Records of the temperature(T)/time(t) behavior of both sample and control are made before, during, and after exposure. From the recorded T(t) versus t during heating and cooling the specific absorption rate (SAR) for microwaves (or the equivalent energy absorption during conventional heating) can be determined. The exposure system consists of two shorted sections of S-band rectangular waveguide with matched coaxial feeds placed in a CO_2 tissue culture incubator thermostated at 37.5°C. Sample holders are supported centrally in the waveguide in blocks of low-density expanded polystyrene. An oscillator, TWT amplifier, isolator, and forward and reflected power meters, provide the power to the exposure waveguide. The second waveguide houses the control samples. Temperatures in one sample chamber in each waveguide are recorded with a Narda Model 8011B non-perturbing probe. The temperature readings are digitized and stored in a desktop computer. Subsequent linear least squares analysis of T(t) versus t with the assumption of constant power absorption and Newton's law of cooling provides a very reliable value for the SAR. On-line determination of the SAR in this way allows accurate assessment of exposure uniformity from one sample chamber to the other, and permits accurate calibration of the system in terms of a SAR/forward-power ratio.

PW-06 INTENSITY DEPENDENCE OF THE INDUCTION OF ACETYLCHOLINE ESTERASE ACTIVITY BY MODULATED RADIOFREQUENCY RADIATION. S. K. Dutta, B. Ghosh*, C. F. Blackman+, Botany Department and Cancer Research Center, Howard University, and +Health Effects Research Laboratory, USEPA, Res. Tri. Pk., NC.

Radiofrequency radiation (RFR) at 915 and at 147 MHz, when sinusoidally amplitude modulated (AM) at 16 Hz, has been shown to enhanced the release of calcium ions from neuroblastoma cells in culture. The dose response for this effect is highly unusual, consisting of two power density "windows" in which enhanced efflux occurs, separated by power density regions where no effect is observed. A similar result has also been reported for a chicken brain-tissue preparation. In order to explore the physiological importance of these findings, we have examined the impact of exposure on a membrane-bound enzyme, acetylcholine esterase (AChE), which is intimately involved with the acetylcholine neurotransmitter system. Neuroblastoma cells (NG-106), exposed for 30-minutes to 147-MHz radiation, AM at 16 Hz, demonstrated enhanced AChE activity, as assayed by a procedure using ^{14}C-labeled acetylcholine. This enhanced activity was observed only within a narrow time window several hours after the cells were seeded, and only when the exposure occurred at power densities that were effective for calcium ions. This result indicates that RFR can perturb nervous-system-derived cells in culture to affect both calcium ion release and AChE activity in a common dose-dependent manner. Both the physiological significance and the underlying mechanism of action of these low intensity fields will need to be reviewed in light of these findings. We acknowledge support from an Institutional Training Grant from Howard University, Cooperative Agreement CR812100 from USEPA, and Interagency Agreement DE-AI06-87RL11374 from DOE.

Attachment #4

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● *Paper*

AN AUTOMATED DOSIMETRY SYSTEM FOR MICROWAVE AND THERMAL EXPOSURE OF BIOLOGICAL SAMPLES *IN VITRO*

Kenneth H. Joyner,* Christopher C. Davis,† Edward C. Elson,‡ Ewa M. Czerska† and Przemyslaw Czerski§

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Abstract—A waveguide exposure system with automated sample temperature measurement is described. This system provides on-line determination of the temperature profile over time of biological samples *in vitro*. It allows automated computation of the specific absorption rate determined from heating/cooling curves, uses minimally-perturbing thermometry, is biocompatible and can be used for measurements of both microwave and conventional heating.

INTRODUCTION

DIELECTRIC HEATING is a recognized mechanism for the induction of radiofrequency (RF) bioeffects considered to be thermal in origin—that is, attributable to temperature increases. However, apart from RF-heat cell killing (Sapareto et al. 1982; Chang et al. 1987), very little information is available on quantitative relationships between RF bioeffects at the cellular and molecular level and the temperature profile over time (TPT). The determination of RF heating/cooling curves can be used to compute the specific absorption rate (SAR W kg^{-1}) and the specific absorption (SA J kg^{-1}) in an exposed sample (Stuchly and Stuchly 1986). Biological variables and exposure conditions can be controlled in experiments with *in vitro* systems to a degree not achievable *in vivo*. An attractive model to study RF bioeffects is the transformation of lymphocytes *in vitro*, provided the biological variables can be related to dosimetric quantities that characterize exposure (Czerski 1975; Budd and Czerski 1985). To accomplish reliable dosimetry, we designed an exposure system with provision for real-time temperature monitoring with RF-field non-perturbing temperature probes. The exposure system, which will be described in detail later, has multiple sample chambers. To allow on-line thermometry and dosimetry, one of these chambers is used as a site for a non-perturbing temperature probe. From the temperature (T)/time (t) history of the sample chamber, an exposure dosage can be determined and described in terms of TPT, SAR, SA, or electric field strength. The system can be used for studies of RF bioeffects in any tissue culture cell line or other *in vitro* biological samples. The temperature monitoring part of the system and the associated automated data analysis can be applied to the study of temperature-dependent effects, irrespective of the modality used for heating.

EXPOSURE SYSTEM

A schematic diagram of the system for microwave exposure of samples is shown in Fig. 1. The output from a CW oscillator* feeds a travelling-wave-tube (TWT) amplifier¶. Pulsed or amplitude-modulated waveforms are obtained through the use of a p-i-n modulator and driver#. For high peak power, but low duty-cycle, pulsed exposures the signal can be further amplified with a 1 kW amplifier**. The amplified signal enters a shorted section of S-band rectangular waveguide 300 mm long through a matched coaxial feed. The waveguide sample holder receives its power through an isolator and a dual-directional coupler that allows forward and reflected powers to be measured with power meters†† and waveforms to be monitored with a diode detector‡‡ and oscilloscope. A sample holder for biological specimens is supported cen-

* Hewlett-Packard Model 8616A, Hewlett Packard Co., 3155 Porter Drive, Palo Alto, CA 94304
¶ Hughes Model 1177H, Hughes Aircraft Co., Electron Dynamics Div., P.O. Box 2999, Torrance, CA 90509.
# Hewlett-Packard Model 8403A.
** MCL Model 10284, MCL, La Grange, IL 10284
†† Hewlett-Packard Model 432A
‡‡ Hewlett-Packard Model 423B.

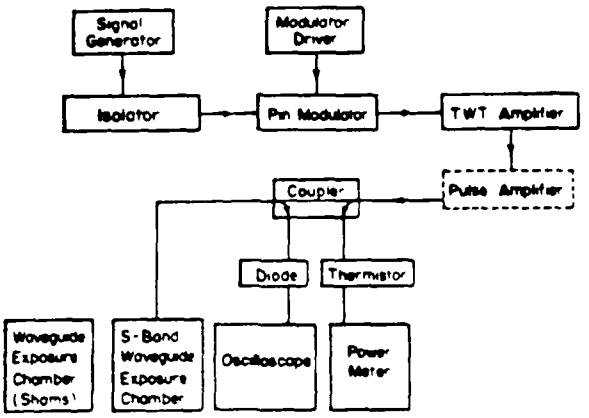

Fig. 1. Schematic diagram of essential components of microwave exposure system.

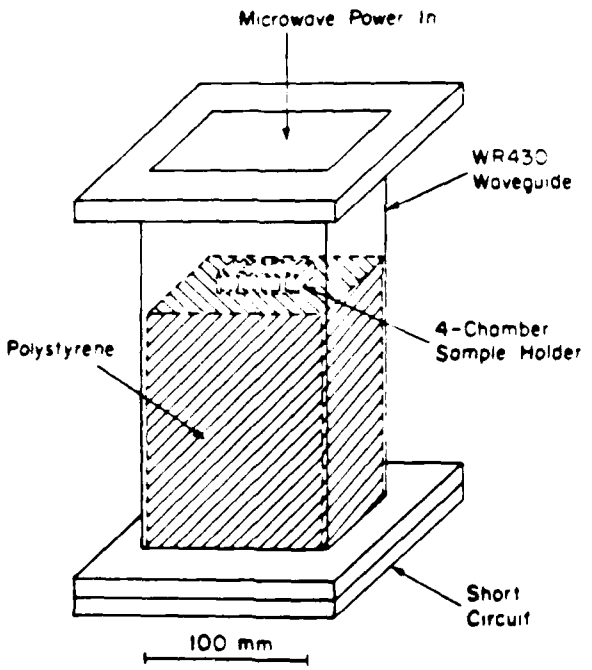

Fig. 2. Waveguide exposure assembly showing location of four-chamber sample holder.

trally in the waveguide in a block of low-density polystyrene that fills the cross-section of the guide, as shown in Fig. 2. An identical waveguide section with an identical plastic sample holder is used for sham exposures. During microwave exposures, both waveguide assemblies were

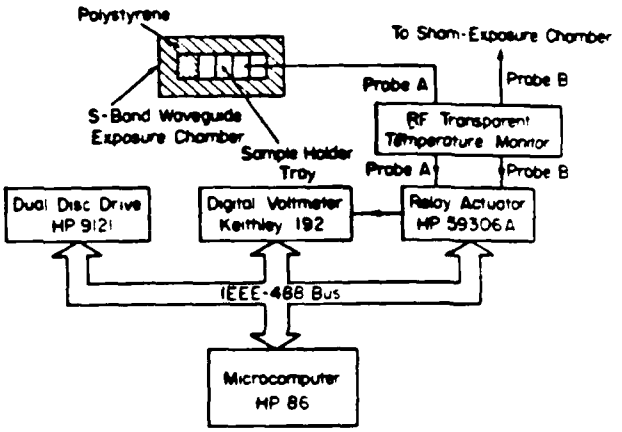

Fig. 3. Schematic diagram of four-chamber sample holder with minimally-perturbing temperature measurement and automated dosimetry system.

housed in a CO_2 tissue culture incubator thermostated at 37.0°C. Various sample holders were tested: a four-chamber plastic tissue culture slide§§, shown in detail in Fig. 3, was found satisfactory in our application. Each chamber holds 1 mL. Since this sample holder occupies only about 10% of the cross-sectional area of the waveguide, field uniformity from one chamber of the sample holder to the other should be within about ±6% of the average and power densities should be within ±12% of the average. This expected exposure uniformity was borne out by quantitative dosimetry. A miniature thermistor temperature probe enters each waveguide section through a small hole in the top shorting plate. The hole is drilled near the guide wall at the center of the shorter dimension. This is a low electric field point, and the hole produces minimal disturbance. The active end of the thermistor probe enters the sample in one of the four chambers of the tissue culture dish. We could move the probe from chamber to chamber to check exposure uniformity. Two types of minimally-field-perturbing probe have been used in this way: a Narda Model 8011B non-perturbing double temperature probe[‡‡] or two Vitek Model 101 probes[¶¶]. The temperatures in both the exposed and sham-exposed samples are recorded continuously during an experimental run. The temperature probes are connected to a relay activator[##] which is connected to a digital voltmeter (DVM)[***]. Both the relay activator and DVM are under the control of a desk-top computer[†††] through the IEEE-488 bus. Under computer control, the temperatures of exposed and sham-exposed samples are recorded sequentially and stored in memory. Since the temperature of sham-exposed samples is essentially constant, it is mon-

§§ Miles Scientific # 4804, Miles Scientific, Division of Miles Laboratory Inc., Naperville, IL 60566

‡‡ The Narda Microwave Corporation, 435 Moreland Road, Hauppauge, NY 11788

¶¶ Available from BSD Medical Corp., 420 Chipeta Way, Salt Lake City, UT 84108

Hewlett-Packard Model 59306A.

*** Keithley Model 192, Keithley Instruments, Inc., 28775 Aurora Road, Cleveland, OH 44139.

††† Hewlett-Packard Model 86.

itored less frequently than the temperature of the exposed samples. A typical experimental protocol would involve 10 temperature readings of the exposed sample for every one reading of the sham-exposed. For conventional thermal exposures, the temperature can be elevated by increasing the temperature of the incubator.

TEMPERATURE/TIME ANALYSIS

During a typical experiment, the temperature is recorded at regular intervals, with a minimum measurement interval of <1 s before, during and after the exposure. The "on" and "off" times of exposure are recorded on the computer by the operator using a "soft-key" interrupt capability. At the conclusion of a run, the $T(t)$ behavior is analyzed to determine SAR.

If the SAR is S (W kg^{-1}) and the specific heat of the sample is C (J kg^{-1} K^{-1}), then the rate of heating during microwave exposure, for small temperature variations over which C can be assumed to be constant, is

$$\left(\frac{dT}{dt}\right) = \frac{S}{C}. \tag{1}$$

If, in the absence of deliberate heating, a sample is above or below its equilibrium temperature, T_{eq}, with its surroundings, then for small temperature differences from equilibrium, its natural rate of temperature change, from Newton's law of cooling, is of the form

$$\left(\frac{dT}{dt}\right) = \alpha(T_{eq} - T), \tag{2}$$

where α is a constant that depends on sample geometry, insulation, etc. In general, when microwave power is applied to a sample that was not in thermal equilibrium with its surroundings at the start of exposure:

$$\frac{dT}{dt} = \frac{S}{C} + \alpha(T_{eq} - T). \tag{3}$$

The solution to eqn (3) can be written in the form

$$T - T_0 = \left(\frac{S}{\alpha C} + T_{eq} - T_0\right)(1 - e^{-\alpha t}), \tag{4}$$

where T_0 is the control temperature at $t = 0$. Either eqn (3) or eqn (4) can be used to determine the SAR. From eqn (3) we have

$$\left(\frac{dT}{dt}\right)_S - \left(\frac{dT}{dt}\right)_{S=0} = \frac{S}{C}. \tag{5}$$

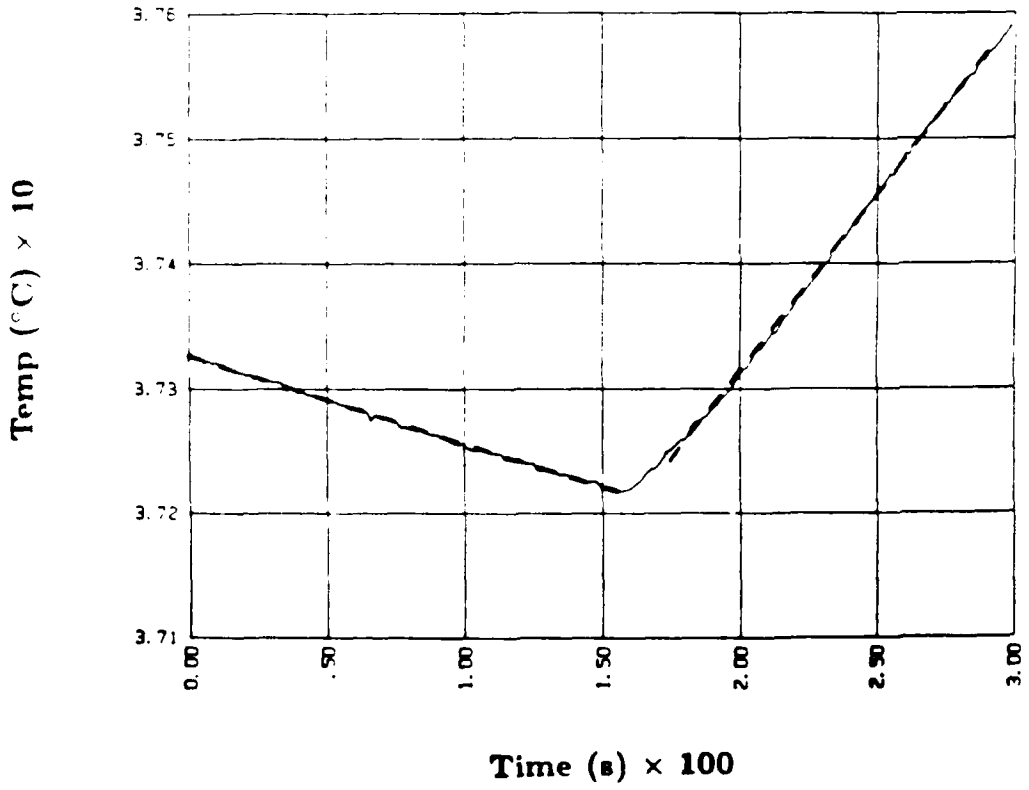

Fig. 4. Temperature/time profile of a sample that was subjected to RF heating (solid curve). The dashed curves are least-squares linear fits to the experimental data. To the left of the time at which RF power was applied, the sample was cooling. The change in slope at the initiation of the RF heating provides the quantitative dosimetry.

where subscript S indicates the application of microwave power corresponding to SAR S. Therefore, determination of the rate of temperature change before and during application of microwave power, or during and after the application of microwave power, determines the SAR.

If the sample is in thermal equilibrium before exposure begins, eqn (4) reduces to the simpler form

$$T - T_0 = \frac{S}{\alpha C}(1 - e^{-\alpha t}), \quad (6)$$

which can be used to find S from $T(t)$. In general, the approach using eqn (5) is preferable, since for times near $t = 0$, the exponential can be expanded in quadratic form.

In practice, this analysis is performed automatically. At the end of a run, the point of inflection, or turning point, in $T(t)$ is found numerically. This is done by checking the change in slope of the temperature as a function of time. In practice, this change in slope occurs immediately upon the application or removal of microwave power, within the time resolution of the data acquisition system. The temperature profile to the left and right of this point is fitted by least-squares to a linear or quadratic function, and the change in slope at the beginning of the exposure period yields the SAR. Figures 4 and 5 are examples of such a procedure. Figure 4 shows the temperature/time history of a sample that was cooling prior to the start of microwave exposure. The linear fits to the cooling and heating portions of the curve yield the SAR. Figure 5 shows the heating of a sample that was quite well equilibrated before exposure. Because the signal/noise ratio is high, the SAR was determined by a quadratic fit to the heating portion of the curve. The quality of the fit near the turning point can always be examined to determine whether the slope value is realistic. However, unless the temperature/time profile has very low noise, it is generally better to use the linear fit to $T(t)$ near the turning point, otherwise a biased estimate of the slope can result. Figure 5 also shows the temperature/time history of the sham-exposed sample to illustrate its temperature stability.

RESULTS AND DISCUSSION

The advantages of the procedure described above are severalfold. The sample need not be equilibrated before SAR is determined. Repeated determination of SAR can easily and quickly be made, which allows the SAR uniformity from one sample chamber to another be deter-

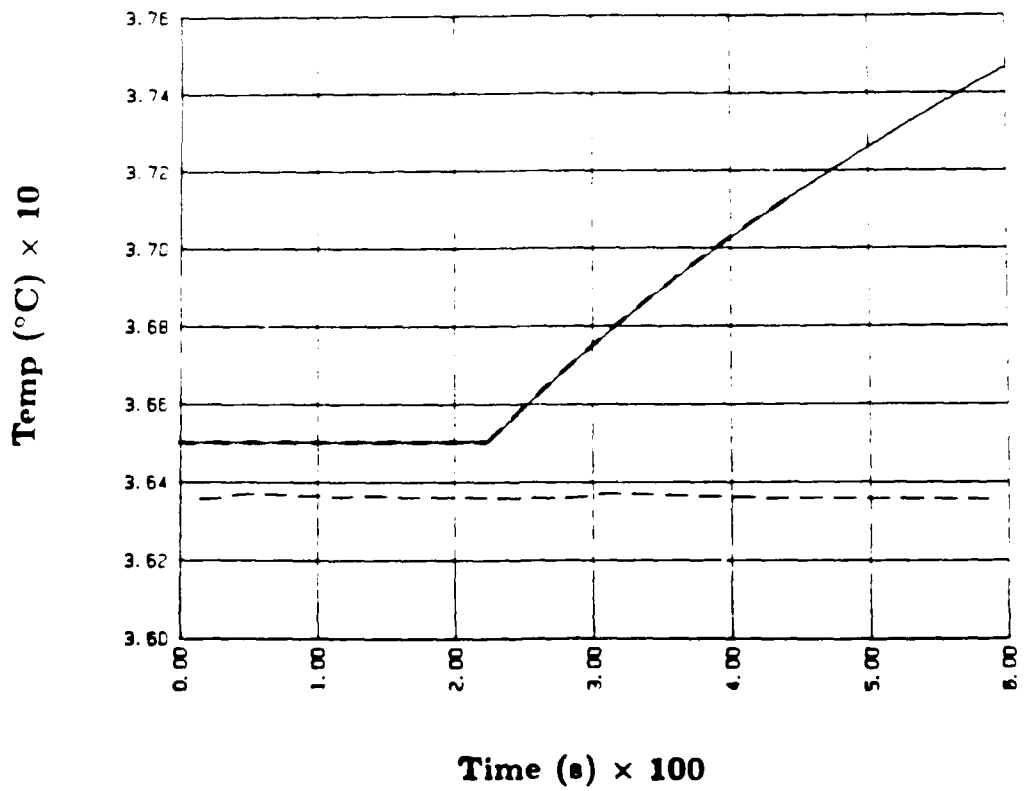

Fig. 5. Temperature/time profile of exposed (upper solid curve) and sham-exposed samples (lower dashed curve). The dosimetry for the exposed sample has been determined from a quadratic fit to the heating curve during microwave exposure and a linear fit to the equilibrated portion of the curve prior to exposure (dashed upper curve).

mined. Exact knowledge of the microwave power is not required; reproducible exposures can be made at known SAR by using the measured forward power corresponding to a given SAR measurement, provided the experimental arrangement is not altered between exposures. Dosimetry is not affected by other losses in the system. The waveguide exposure system with four sample chambers was found to give an SAR uniformity from one chamber to the other within 10%. Thus, experimental samples can be exposed and examined in triplicate, the fourth chamber being used for the insertion of the temperature probe.

The system is biocompatible and, depending on cell line, cell density and medium, allows continuous exposures of several days duration. The biocompatibility of this arrangement was proven by studying the growth of human lymphocytes under various conditions within the waveguide. A concentration of 10^6 cell mL^{-1} in chromosome medium 1A‡‡‡ with or without phytohemagglutin was incubated for 72 or 120 h in the exposure system with no applied RF power. Cell morphology, lymphoblastoid transformation, mitotic index and cell viability tested by neutral red, Janus green and trypan blue stains did not differ from those in lymphocyte cultures from the same donor cultured in a conventional CO_2 incubator according to the protocol provided by Gibco with their media. As well as providing biocompatible exposure conditions, the system is flexible. It allows the exposure of tissue culture cells growing in suspension or in monolayers and can easily be used with cell-free samples.

The exposure system is relatively simple and can be assembled from off-the-shelf components. Its capabilities can be expanded by introducing computer control of RF power input based on feedback from temperature measurements in a similar way to the system described by Chang et al (1987). This system used a stripline operating at 915 MHz and allowed uniform exposure of 10 mL tissue culture samples. The temperature/time profile of heating could be pre-programmed, and biocompatibility allowed exposures of several days duration. A. W. Guy (1977) designed a system for exposure of 5 mL samples in the range from dc to 1 GHz. Field strength dosimetry was obtained from on-line measurement of feed-line impedance. The temperature of the sample could be maintained at a preset level using a circulating liquid heat exchanger. No data on biocompatibility of this system were provided, however the information given suggests suitability only for short-term exposures. J. C. Lin (1976) and Chen and Lin (1978) proposed the use of micropipettes immersed in a fluid-filled waveguide irradiation chamber for *in vitro* studies under controlled temperature and dosimetric conditions. This system was also only suitable for short-term exposures. J. W. Allis et al. (1975) installed a waveguide exposure system in a Cary 15 spectrophotometer allowing simultaneous exposure and spectrophotometric biochemical determinations. Our system, as well as those described in the papers quoted here, provides a wide range of possibilities for *in vitro* studies under controlled conditions, including precise RF dosimetry. By using one of these systems, or combining desired features of some of them, most requirements for the study of RF bioeffects at the cellular and/or molecular level can be met.

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‡‡‡ Gibco Laboratories, 3175 Staley Road, Grand Island, NY 14072.

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